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## A CLINICAL PATHOLOGIST FOR EVERY APPROVED HOSPITAL\*

PHILIP HILKOWITZ

Considerable criticism has been leveled against the medical profession for its alleged failure to provide proper medical care for the entire population. Physicians have also been accused of not doing their full duty in the field of prevention of disease, leaving the task mainly to lay sanitarians. Whether these strictures are justifiable or not is outside the scope of this paper. I shall limit my inquiry to the rôle of the clinical pathologist in the general health program and the power inherent in the American Society of Clinical Pathologists to extend his usefulness.

It would be superfluous to dwell on the importance of laboratory methods in the practice of medicine as their use has already received universal recognition and they are now considered indispensable both in hospitals and private practice. How far, however, have the services of the clinical pathologist been utilized in the care of patients throughout this country? What can our Society do to stimulate a wider employment of his talent?

The medical profession has been reproached for not taking a positive attitude and greater initiative in spreading the benefits of scientific medicine to all strata of society. As specialists in one of its essential branches, the American Society of Clinical Pathologists should abandon a *laissez faire* policy and endeavor to devise ways and means whereby the benefits of the test tube and microscope may be more widely diffused than they are at present. Many elements of our citizenship are still deprived of the counsel of the clinical pathologist in the diagnosis and treatment of disease. The lack is by no means limited to the poor

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but, as will be shown in our study of hospitals, it affects likewise the rich and the middle class.

The enormity of the task of ascertaining the degree of utilization of scientific medicine in the practice of medicine made it advisable to narrow the inquiry to hospitals, and to leave out for the present the care of the sick in the home or office.

With the aid of data kindly furnished by the office of the American College of Surgeons, supplemented by a check-up on the part of our State Counsellors, a preliminary survey has been made of the hospitals of the United States with particular reference to the conduct of their respective clinical laboratories under the supervision of a clinical pathologist. No personal inspection was made of the institutions as to the extent and character of such supervision, all inquiries being directed by mail through questionnaires. I take this opportunity to thank the State Counsellors of the A. S. C. P. for their generous coöperation which was cheerfully given at the expenditure of valuable time and labor, not to mention the cost of postage and clerical help.

The study did not include government hospitals nor institutions for the insane, and likewise omitted many state institutions and sanatoria, all of which in the aggregate loom large in the care of patients and form a factor in discussions on socialization of medicine. I am keenly conscious of the defects inherent in this sketchy survey and hope that it may serve as a stimulus for the American Medical Association or the American College of Surgeons to undertake this task on a more comprehensive scale. Such data are already available in the archives of the Council on Medical Education and Hospitals of the American Medical Association and need only collation, which it is hoped will be effected in the near future.

Let us now look at table 1 which shows the relative number and percentage of hospitals for each state which are under more or less supervision by a clinical pathologist. Be it remembered that in quite a number of hospitals his direction is rather nominal and consists solely of interpreting such tissues as the administration or the staff choose to send to him. It is my firm conviction that a clinical pathologist should not consent to serve any hospital

TABLE 1  
EXTENT OF SUPERVISION OF HOSPITALS IN THE UNITED STATES BY CLINICAL  
PATHOLOGISTS

STATE	TOTAL NUMBER OF HOSPITALS	WITH CLINICAL PATHOLOGISTS	PERCENTAGE	APPROVED HOSPI- TALS	WITHOUT CLINICAL PATHOLOGISTS	PERCENTAGE	BED CAPACITY		
							Under 50	50-100	Over 100
Alabama.....	40	14	35.0	23	10	43.4	9	22	9
Arizona.....	22	7	31.5	10	8	80.0	8	10	4
Arkansas.....	27	12	44.5	16	8	50.0	10	7	8
California.....	167	96	57.4	98	9	9.1	40	34	74
Colorado.....	40	29	72.5	36	7	19.4	7	10	21
Connecticut.....	32	26	81.5	28	3	10.7	4	6	20
Delaware.....	8	2	25.0	6	0		2	2	4
District of Columbia.....	15	15	100.0	15	0			2	13
Florida.....	43	29	67.4	27	7	25.9	16	16	10
Georgia.....	55	16	29.8	30	17	56.6	23	18	11
Idaho.....	22	15	68.1	8	1	12.5	13	5	2
Illinois.....	177	104	58.7	123	22	17.8	29	53	90
Indiana.....	78	22	28.2	35	19	54.2	30	16	28
Iowa.....	71	23	32.3	45	21	46.6	26	23	22
Kansas.....	61	17	27.8	43	27	62.7	18	21	16
Kentucky.....	53	8	15.0	25	19	76.0	20	20	12
Louisiana.....	32	16	50.0	23	8	34.7	10	8	13
Maine.....	32	9	28.1	20	11	55.0	13	9	9
Maryland.....	34	16	47.0	27	9	33.3	7	6	20
Massachusetts.....	141	99	70.2	112	27	24.1	38	37	65
Michigan.....	108	55	50.9	70	21	30.0	44	23	38
Minnesota.....	78	35	44.8	58	24	41.3	33	16	29
Mississippi.....	49	3	6.1	27	25	92.5	22	19	7
Missouri.....	81	54	66.6	64	14	21.8	13	18	45
Montana.....	26	10	38.4	20	10	50.0	7	11	6
Nebraska.....	32	16	50.0	25	10	40.0	6	5	20
New Hampshire.....	26	11	42.3	19	10	52.6	11	13	2
New Jersey.....	87	61	70.1	68	11	16.1	15	17	55
New Mexico.....	14	5	35.7	8	6	75.0	6	5	3
New York.....	310	194	62.5	233	59	25.3	44	90	165
Nevada.....	5	1	20.0	3	2	66.6	4	1	0
North Carolina.....	99	22	22.2	61	40	65.5	51	30	16
North Dakota.....	21	12	57.1	17	12	70.5	6	9	6
Ohio.....	133	73	54.8	93	24	25.8	39	34	54
Oklahoma.....	56	10	17.8	25	18	72.0	21	20	10
Oregon.....	36	22	61.9	21	8	38.0	10	19	6
Pennsylvania.....	209	133	63.6	169	45	26.6	24	75	107



TABLE 1—*Concluded*

STATE	TOTAL NUMBER OF HOSPITALS	WITH CLINICAL PATHOLOGISTS	PERCENTAGE	APPROVED HOSPI- TALS	WITHOUT CLINICAL PATHOLOGISTS	PERCENTAGE	BED CAPACITY		
							Under 50	50-100	Over 100
Rhode Island.....	11	9	81.8	11	2	18.1		2	9
South Carolina.....	33	9	27.2	15	6	40.0	13	12	7
South Dakota.....	26	6	23.0	21	17	80.9	8	14	3
Tennessee.....	54	29	53.7	40	9	22.5	23	13	16
Texas.....	120	59	49.1	41	41	100.0	48	30	37
Utah.....	10	3	30.0	6	4	66.6	3	1	6
Vermont.....	16	6	37.4	12	6	50.0	6	7	3
Virginia.....	63	26	41.1	43	22	51.1	18	27	16
Washington.....	51	42	82.5	39	3	7.6	15	12	22
West Virginia.....	58	24	41.3	39	17	43.5	14	24	17
Wisconsin.....	87	32	36.9	48	16	33.3	32	22	33
Wyoming.....	14	3	21.4	8	5	62.5	8	3	2
Totals.....	3,063	1,540		2,054	720		867	897	1,191

(Note: There were 108 hospitals on which no data were available covering the bed capacity.)

The 108 Hospitals for which no bed capacity data were available were distributed as follows:

Arkansas.....	2	Montana.....	2
California.....	19	Nebraska.....	1
Colorado.....	2	New York.....	11
Connecticut.....	2	North Carolina.....	2
Florida.....	1	Ohio.....	6
Georgia.....	3	Oklahoma.....	5
Idaho.....	2	Oregon.....	1
Illinois.....	5	Pennsylvania.....	3
Indiana.....	4	South Carolina.....	1
Kansas.....	6	South Dakota.....	1
Kentucky.....	1	Tennessee.....	2
Louisiana.....	1	Texas.....	5
Maine.....	1	Virginia.....	2
Maryland.....	1	Washington.....	2
Massachusetts.....	1	West Virginia.....	3
Michigan.....	3	Wyoming.....	1
Mississippi.....	1	Total.....	108
Missouri.....	5		



unless he be invested with full responsibility for the conduct of the laboratory and choice of the personnel. If on a part time basis, he should visit the institution at periodic intervals and participate in the monthly staff meetings. He should, therefore, be held accountable for any shortcomings of his own or of the technicians under him. A hospital that merely sends its tissues to a pathologist and puts the laboratory in charge of a technician is not doing its proper duty to the sick nor to its medical staff, and should not be accepted for approval. The same table also enumerates for each state the total number of hospitals, their classification as to bed capacity, the actual number of those served by a pathologist, and their respective percentage, also the number of approved hospitals and the number and per cent not supervised.

Without delving too deeply into the detailed figures we may draw up the following summary:

1. Of the nearly 3000 hospitals in the United States, approximately one-half (46.9 per cent) are functioning without a clinical pathologist.
2. Roughly one-third of the hospitals approved fully or provisionally by the American College of Surgeons do not conform to its recommendation of having the laboratory under the direction of a physician-pathologist.

As to small hospitals, say under twenty-five beds, I hazard the opinion that, except in isolated communities, they should not be encouraged but should be absorbed by larger institutions. They are mostly operated by individual physicians, cannot afford even a technician, and often are merely boarding houses with the fancy title of hospital. In this age of rapid transportation and with communities establishing their own institutions, or religious organizations with their long experience founding large hospitals, the dissipation of energy into small units is contrary to efficiency and proper planning.

Are we ready to furnish proper service in the places now vacant of scientific guidance? Table 2 shows the distribution of clinical pathologists in the United States. The figures are derived from the roster of membership of the American Society of Clinical

TABLE 2  
DISTRIBUTION OF CLINICAL PATHOLOGISTS IN THE UNITED STATES

Alabama.....	4	Nevada.....	1
Arizona.....	3	New Hampshire.....	1
Arkansas.....	5	New Jersey.....	30
California.....	60	New Mexico.....	1
Colorado.....	18	New York.....	170
Connecticut.....	11	North Carolina.....	10
Delaware.....	1	North Dakota.....	3
District of Columbia.....	13	Ohio.....	40
Florida.....	6	Oklahoma.....	12
Georgia.....	9	Oregon.....	8
Illinois.....	53	Pennsylvania.....	119
Indiana.....	15	Rhode Island.....	4
Iowa.....	14	South Carolina.....	7
Kansas.....	6	South Dakota.....	1
Kentucky.....	9	Tennessee.....	11
Louisiana.....	21	Texas.....	36
Maine.....	4	Utah.....	3
Maryland.....	8	Vermont.....	1
Massachusetts.....	32	Virginia.....	10
Michigan.....	34	Washington.....	13
Minnesota.....	19	West Virginia.....	9
Mississippi.....	3	Wisconsin.....	17
Missouri.....	24	Idaho.....	0
Montana.....	4	Wyoming.....	0
Nebraska.....	8	Total.....	881

STATE	NUMBER OF HOSPITALS	APPROVED HOSPITALS	CLINICAL PATHOLOGISTS
Alabama.....	40	23	4
Arizona.....	22	10	3
Arkansas.....	27	16	5
California.....	167	98	60
Colorado.....	40	36	18
Connecticut.....	32	28	11
Washington, D. C.....	15	15	15
Delaware.....	8	6	1
Florida.....	43	27	6
Georgia.....	55	30	9
Idaho.....	22	8	0
Illinois.....	177	123	53
Indiana.....	78	35	15
Iowa.....	71	45	14
Kansas.....	61	43	6
Kentucky.....	53	25	9

TABLE 2—*Concluded*

STATE	NUMBER OF HOSPITALS	APPROVED HOSPITALS	CLINICAL PATHOLOGISTS
Louisiana.....	32	23	21
Maine.....	32	20	4
Maryland.....	34	27	8
Massachusetts.....	141	112	32
Michigan.....	108	70	54
Minnesota.....	78	58	19
Mississippi.....	49	27	5
Missouri.....	81	64	24
Montana.....	26	20	4
Nebraska.....	32	25	8
Nevada.....	5	3	1
New Hampshire.....	26	19	1
New Mexico.....	14	8	1
New Jersey.....	87	68	30
New York.....	310	233	170
North Carolina.....	99	61	10
North Dakota.....	21	17	3
Ohio.....	133	93	40
Oklahoma.....	56	25	12
Oregon.....	36	21	8
Pennsylvania.....	209	169	119
Rhode Island.....	11	11	4
South Carolina.....	33	15	7
South Dakota.....	26	21	1
Tennessee.....	54	40	11
Texas.....	120	41	36
Utah.....	10	6	3
Vermont.....	16	12	1
Virginia.....	63	43	10
Washington.....	51	39	13
West Virginia.....	58	39	9
Wisconsin.....	87	48	17
Wyoming.....	14	8	1

Pathologists as published in the November 1935 number of our JOURNAL, and also from the list of approved clinical pathologists issued by the Council on Medical Education and Hospitals of the American Medical Association (October 26, 1935). Care was exercised to eliminate duplication of names. The total for the Union is eight hundred and eighty-one (881). Doubts have been expressed whether all of them are competent to serve as hospital

pathologists with all the attributes that this implies, especially in the field of tissue diagnosis. With the institution of the projected Board examinations we may expect more exact data as to the competence of the specialists in this field.

Be that as it may, and accepting the present total of clinical pathologists as a correct index, we may safely assume that, given a proper distribution throughout the various states, the hospitals now lacking trained direction could be taken care of by pathologists on a part time basis. For the entire United States, this would constitute one pathologist for every three hospitals which is by no means a difficult task in the efficient supervision of a one-hundred-fifty bed unit. A pathologist in a metropolitan center could also easily direct the hospitals in the neighboring small towns which are now to a large extent neglected. He is in a position to infuse scientific vigor in the staff and bring the rural hospitals up to the level of the big town institution.

The problem of placing a pathologist into at least every approved hospital has a number of ramifications which for lack of space and time cannot adequately be discussed here. The chief impediment is the financial one which, though formidable in appearance, can easily be hurdled. The laboratory can be made self-supporting by judicious management. More difficult by far is the distribution of our men. Here we must have the concerted effort of our State Counsellors backed by the Society and the hearty coöperation and moral support of the American Medical Association and the American College of Surgeons. Both of these powerful national bodies have helped us in the past. Interested as they are in the furtherance of scientific medicine they will continue to exert their influence in this forward movement.

I would, therefore, urge the appointment of a special committee by this Society to bring this project to fruition.

## THE ECONOMIC ASPECTS OF THE HOSPITAL LABORATORY\*

ROBERT A. KILDUFFE

*Director, Laboratory of the Atlantic City Hospital*

In accepting—with some reluctance—an assignment to discuss before this conference some phases of the hospital laboratory and the hospital pathologist, I am well aware that the timidity of the proverbial angel had much to commend it and that, in all probability, I have taken upon myself what may well prove to be a thankless task. For this problem is so involved, embraces so many varied and at times somewhat conflicting interests, and presents so many diverse aspects, that its solution—if one there be—can hardly evolve from a single mind nor emerge clear-cut from a single discussion. Indeed, the most that can first be hoped for is that the problem itself shall become clear-cut, and that it shall be recognized as a problem some solution of which eventually must be found.

It will therefore be my purpose to outline the situation as I see it, to discuss briefly some of the factors which make it a difficult problem, and to present, not only what seem to me to be some of its essential features, but also some purely individual suggestions concerning them; and in so doing no one will realize more clearly than myself that my thoughts on this matter may seem, in the quaint words of Benjamin Franklin<sup>1</sup>, "better calculated to please the fancy than to form the Judgment."

In what follows it is to be understood that we are concerned, not with large institutions blessed with ample endowments, not with world-famed clinics with almost illimitable resources and manned by exceptional personnel, nor with those hospitals whose clinical laboratories are overshadowed by their research divisions.

\* Read before the Fifteenth Annual Convention of the American Society of Clinical Pathologists, Kansas City, Missouri, May 8 to 10, 1936.

On the contrary, we shall be concerned—as we should be—with the average hospital of average size whose clinical laboratory, while neither impressive in extent, equipment, or personnel, carries, nevertheless, a heavy load and—to its credit be it said—still manages to make some contribution to the sum of medical knowledge and advance. It is inescapable that we should be concerned with these for it is in such hospitals that the problems of the laboratory and the pathologist are most acute and most urgently demand consideration.

It seems to me essential to an understanding of this problem that it be seen, first, in perspective as a whole. To this end it becomes necessary, therefore, to consider the functions of the hospital pathologist and the hospital laboratory, the basis for their existence as a part of the hospital, and the real foundation upon which their value to the hospital depends.

As I see it, the fact that it is now mandatory upon hospitals to have a clinical laboratory directed by an accredited clinical pathologist, if they are to attain and maintain an acceptable rating and so be eligible for the training of internes, is largely incidental. For we all know that all too often an acceptable classification signifies simply that the hospital has attained a complacent standard of mediocrity; that, all too often, "class A-dom" signifies merely "lip-service" to set forms and standardized procedures leading to varied degrees of stagnation; and that standardization may be readily degraded to blind and servile adherence to forms and procedures and standardized records with but little care or interest in their real character and real value.

The hospital laboratory, suitably conducted under proper direction, is not only an integral part of the hospital but should be—as it often is—the center of the hospital's activities. For I conceive the function of a hospital to be something more than merely to serve as a place to which the sick and injured may be brought. I believe that the hospital—any hospital, regardless of size—may well be considered, and expected to be, a source for the dissemination and acquisition of medical knowledge; that if it does not teach its staff and its community, and even, perhaps,



add something to the sum of medical advance, even though this be but a widow's mite, it has failed in its responsibilities and its purpose.

To me this does not necessarily connote a "research department" for the development of new discoveries, for just as some of the epoch-making discoveries in medicine have developed from the studies and observations of the general practitioner, so also may something of value emerge from the careful study of accurate and systematic records and from the trial and critical analysis of newer methods developed elsewhere.

All this, I believe, is so relatively obvious that further time need not be spent upon its discussion.

But if the hospital laboratory is important, the pathologist by whom it should be directed is still more so; for in these days it is also rather obvious that the standing of the hospital pathologist and the character and efficiency of the laboratory he conducts—which inevitably must be a reflection of his own—in no small measure may well be indicative of the character and efficiency of the hospital staff in general as well as of the work done in that hospital.

We cannot escape, therefore, in this broad view of the problem in general, some consideration of what shall be the status of the hospital pathologist, for upon him depends primarily the standing of the hospital clinical laboratory and, to some extent, the standing of the hospital itself.

It is no longer necessary to emphasize that the practice of clinical pathology is the practice of a specialized phase of medicine and still less necessary, I hope, to reiterate that its efficient practice demands as much training and experience as is required for any other specialty. For the days when the pathologist was regarded as one who "conducts manipulations on fixtures, inanimate substances" have passed into oblivion and the many ramifications of clinical pathology, as well as its close and interlocking relation to medicine in general—all of which illustrate the degree to which the pathologist must be informed, and the skill and acumen he must develop—should no longer require discussion or elaboration.

But that, oddly enough, it still becomes necessary from time to time to reflect upon it is evidenced by the fact that there are still some hospitals and some hospital administrators to whom the pathologist would appear to bear in some respects the aspect of one of the "hired help," merely a more or less high-grade technical worker laboring in the shadows of an archaic past when the laboratory was a place unacceptable to any other department, and the pathologist, so-called, a man who took the job because it was the only position open, or one who subsided into the laboratory for lack of ability to achieve success in any other field of medicine.

In part, perhaps, this may be due, as Kracke<sup>2</sup> has said, to the fact that "there seems to be some tendency on the part of our clinical colleagues to set laboratory medicine apart from clinical practice and to look upon the average laboratory as a room filled with peculiar, eccentric, scientific people who issue their findings in a systematic manner on pieces of white paper which are attached to patients' charts. This attitude has resulted in some hospital administrations placing the laboratory in a category similar to the drug-room, the diet kitchen, the laundry, and other unscientific divisions of hospital activity."

In this connection I can do no better than excerpt from a letter written by Dr. Herbert Fox<sup>3</sup> in reply, evidently, to an inquiry from one who was considering a directorship of a hospital laboratory.

"A pathologist," he says, "is as much a chief and consultant as any other member of the staff and, consequently, equivalent to any staff chief. The pathologist should be in no way subordinate to the managerial director or the board of directors other than would be the case with a surgeon, a pediatricist, and the like. His duties, if they have to be outlined and interpreted, are professional matters at the discretion of the staff, (and) any member of the staff should be willing to cooperate to the extent that he would be advised and influenced by the staff as far as hospital matters are concerned. . . . Finally, the position of a pathologist must remain a professionally ethical one upon a gentleman's agreement comparable to that holding good with his associates upon the staff."



So much for the problem in its broad and general aspects upon which, I am sure, there will be more or less general agreement. We come now to much more debatable ground upon which unanimous agreement can hardly be expected. But as agreement can only evolve from an expression and comparison of thoughts and ideas, I shall express here—as individual opinions to be taken merely for what they may be worth—my own concepts of the relation which should exist between the hospital and the pathologist.

I. Should the pathologist be a member of the hospital staff?

Emphatically and without qualification—yes, for reasons too obvious to require discussion and which have already been suggested by Dr. Fox.

II. Should the agreement between them be based upon a written contract?

No. First, because, as has just been said, the status of the pathologist is the status of any other staff chief and thus may equitably and fairly rest upon a gentleman's agreement. If either the pathologist or the hospital will not or cannot carry out the spirit of such an agreement, either or both could easily evade or nullify the letter of the contract which thus becomes merely another "scrap of paper."

Second, because a contractual agreement carries with it the concept of employer and employee and thus tends insidiously to relegate the pathologist to the level of one working merely for hire and differing in status from his professional colleagues.

III. How should the pathologist be remunerated?

A. Should he be solely upon a specified salary basis?

This is a matter upon which there is room for much difference of opinion. The answer depends, of course, upon some clarification of the nebulous term "salary."

If the pathologist is devoting his entire time and services to the hospital he may be upon a sole salaried basis without injustice *providing* that: (a) the salary is adequate and in proportion to his skill, experience, and reputation; and, (b) that as his skill, ability, and the degree to which he becomes an asset to the hospital increase in the course of time, there is provision for an increase in salary up to an adequate and reasonable maximum.

In this respect hospitals are somewhat peculiar, for such a provision applies to almost all hospital employees: the superintendent, the operating room supervisor, the housekeeper, and even the elevator man may justly expect some recognition in the shape of salary increase for long and satisfactory service. But many pathologists remain in receipt of the same salary, or practically the same, even though the hospital may have grown, its work increased, and its laboratory expanded many-fold. In fact, instances are not unknown where a pathologist, unwilling to accept such a stagnant, palpably unjust, but apparently irremediable situation, for that reason alone has been replaced by one having the preëminent qualification of being willing to work for less—usually a younger and less experienced man eager to start in somewhere, somehow.

It is obviously difficult to state without qualification what constitutes an adequate salary for a whole-time pathologist of ability and repute, for many factors exert a definite influence.

If it be accepted that the clinical pathologist is, first of all a physician possessing a suitable background of clinical training and experience upon which has been superimposed an ample, extensive, and highly specialized postgraduate training; if it be accepted that in professional standing he differs in no way from any other member of the hospital staff and occupies no lesser status—for indeed it may well happen that his reputation in his own field may be so outstanding and farflung as to compare with that of any other staff member and make him a valuable asset to the hospital *per se*—if these premises be accepted, as I think they may, then it follows that he should receive a fair and equitable compensation, and as Dr. Fox has said, his salary should average the professional income of the rest of the staff.

It should emphatically *not* follow that because, by virtue of his choice of specialty he has of necessity removed himself from the higher income brackets common to other specialties, he should therefore be penalized in the matter of gross income or that, unlike other specialists or practitioners at large, he should be deprived of the expectation that as his skill and experience increase his income should not also.

B. Should the pathologist's remuneration be a percentage of the gross laboratory income, a percentage of the net profits, or a percentage of the gross income minus charity and courtesy allowances?

All of these various methods have been suggested and any of them may be satisfactory *but only if and when the remuneration thus arrived at falls within the provisions outlined above.*

C. Should the pathologist's remuneration be a combination of a basic salary augmented by a percentage of the laboratory gross receipts?

Assuming an adequate basic salary (from \$5,000 to \$6,000) and a percentage of from  $33\frac{1}{3}$  to 50 per cent, this method in my opinion is the most satisfactory and advantageous to both hospital and pathologist.

It assures that both shall share in economic ups and downs; it stimulates the pathologist continually to give his best and allows the hospital to demand this fairly; it encourages the pathologist to remain where he is rather than accept offers which may come to him because of increase in reputation, and thus assures the hospital of the benefit of such increase in skill and experience; and, finally, it does not condemn the pathologist to a static existence not devoid of discouragement and discontent.

IV. Should the pathologist confine himself to institutional work? In other words should he or should he not do laboratory work for, and collect a fee for it, from patients referred for that purpose from outside the hospital?

If, as is sometimes the case, the hospital laboratory is the only laboratory in the community under the direction of a clinical pathologist, then I believe not only that the hospital pathologist should do outside work, but that it is preferable that he should do so rather than have it done in laboratories conducted by lay technicians. Where such a situation does not exist there is a pronounced division of opinion as to the propriety of outside work being done by the hospital pathologist. As is always the case, however, in matters under dispute, there is something to be said on both sides of the question.

On the one hand, it can justly be maintained that the hospital

pathologist doing outside work thereby comes into direct competition with the pathologist conducting a private laboratory who, of course, has what the hospital pathologist does not, namely, the overhead incident to the laboratory maintenance.

On the other hand, in the last analysis it is not the laboratory procedure but its clinical application, and especially its clinical significance, which is of paramount importance. The wise clinician is therefore more often interested in the pathologist's choice of laboratory procedure and his opinion as to the significance of its results than in the procedure or its results *per se*. For this reason staff men may be prone to send their laboratory work to the hospital laboratory because of the ease with which they may consult the pathologist and especially because, by virtue of their association with him and the opinion they have formed of his ability, it is *his* opinion they particularly seek and desire.

Under these circumstances it is somewhat difficult for the hospital pathologist to avoid such work without embarrassment or offense. It must either be done without charge, which in many instances is not only unjustified and in no way lessens but rather aggravates the element of competition; or it can be charged for, which leaves the problem unchanged.

An additional factor enters the situation where the pathologist is remunerated by an inadequate salary alone which must be supplemented if he is to exist, as he and his family are entitled to do so that he may thereby be forced as it were into outside work. If his salary or remuneration, however obtained, is on a fair and equitable basis, then certainly he should not deliberately engage in active competition with his fellows, although, as has been suggested above, it may be difficult to avoid it in some small measure.

Unfortunately, a still further complication arises from the fact that not a few hospitals look largely to outside laboratory work as the source from which a part, at least, of the laboratory maintenance must come. To that degree the hospital is in direct competition with the private clinical pathologist, but as things now are the hospital is in direct, active, and even aggressive

competition with the physician at large—regardless of his specialty—the end of which no man forseeth, least of all myself.

Certainly, however, this much can be set forth as an incontrovertible principle which should be inviolable: the fees for outside work done in the hospital laboratory should be the same as those applying throughout the community. The hospital laboratory should no more do “cut-rate” laboratory work than the surgical staff do “bargain” operations, or the medical staff “cut-rate” consultations, or the hospital itself offer “cut-rate” accommodations. If competition there must be, it should at least be on a fair and honest basis and depend more upon ability and reputation than upon access to equipment and facilities. For, as I have already indicated, what is being bought, in the last analysis, is the right to secure, when desired, the pathologist’s opinion, and his interpretation of the results of the laboratory examination.

It is apparent, therefore, that when outside work is done in the hospital laboratory the fees to be charged are a matter to be determined, not by hospital administrators, but solely and entirely by the pathologist, or, if so desired, by the pathologist and the staff in conference.

V. Should the pathologist share in the laboratory profits, if any?

If on a salary basis alone, and particularly when the salary is less than the responsibilities of the position entail—yes.

The laboratory profits rise from the volume of work done. This is influenced by the reputation of the hospital which, in turn, is dependent upon the skill and reputation of its staff—of which the pathologist is a not insignificant member. For these reasons he should be entitled to some share of the hospital’s prosperity just as its surgeons and physicians secure added and profitable work from their association with the hospital, in the same way that the hospital secures a proportion of its private and income-producing patients because of the skill and reputation of its staff.

VI. What should be the status of the pathologist as a consultant in the hospital?

Few will deny that the pathologist exerts his most important



and valuable function when he acts as a liason officer between the laboratory and the bedside. It is to be expected, indeed demanded of him, that he be competent to bring a definite contribution to the study of a puzzling or obscure case, whether in suggesting the most appropriate and most likely to be informative avenues for its laboratory investigation; in shedding light upon the minutia of their clinical significance; or as competent and skilled in the utilization of certain specialized methods of treatment.

He is used, therefore, or should be, in a consultant capacity in exactly the same manner as any other staff man and, as a consultant, he should occupy the same status as any other and charge consultant fees to private, paying patients. And as such consultations arise, not from his hospital position but from his personal ability and reputation, such fees should justly be his alone.

In thus presenting to you my own views upon what is, and cannot help but be, a controversial subject, I appreciate that I have but scratched the surface of its complexities. It was neither my intention nor my hope to bring to this conference a fully developed or completely satisfactory solution of this grave and even acute problem for, in the words of Disraeli, "it is much easier to be critical than correct."

Nor do I expect any unanimous agreement upon the propositions I have laid down nor even, perhaps, upon my concept of the problem itself; for where there are many men there are many minds wherewith to draw diverse conclusions. And "who shall decide when doctors disagree?"

If it be objected, as it may, that I have presented, and perhaps even emphasized, the financial phases of the problem, I can but reply that pathologists are not lilies of the field, and if they and their families are to have food and clothes and even some measure of the comforts of life, I see no reason why these should be sued for in *forma pauperis*. For, as Shakespeare says, "I am as poor as Job, my Lord, but not so patient."

If I have achieved anything by this discussion it may be that I have presented as clearly as I could, not the solution of a prob-

lem, but the problem itself for consideration; for, in the words of General Wolfe in one of his dispatches to Lord Pitt: "there is such a choice of difficulties that I find myself at a loss how to determine."

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## EFFICIENCY OF STATE AND LOCAL LABORATORIES IN THE PERFORMANCE OF SERODIAGNOSTIC TESTS FOR SYPHILIS\*†

THOMAS PARRAN

*Surgeon General, United States Public Health Service, Chairman‡*

More than a year ago the Committee on Evaluation of Serodiagnostic Tests for Syphilis reported the results of its first study.<sup>1</sup> This report recorded the ability of several of the outstanding serologists in the United States to perform tests which they had originated, or to perform pre-existing tests which they had modified. The efficiency of the performance of the tests of these serologists was evaluated on the basis of sensitivity and specificity. In the conclusions of the report, the Committee stated that it recognized that the actual serologic testing had been performed under relatively ideal conditions and furthermore, that the results did not necessarily compare with those obtained from the performance of these serologic methods in other laboratories.

In the summer of 1935, notices were published in leading medical journals of the country in which a project was described, the object of which was the evaluation of the reliability of the different serodiagnostic tests for syphilis as performed in State and local laboratories. A number of directors of such laboratories who expressed an interest in the proposed study were invited to participate and those invited were later requested to designate

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† Note: Copies of the Detailed Report of Results of the Evaluation of Serodiagnostic Tests for Syphilis in the United States (Supplement No. 1 to Ven. Dis. Inform.) are available from the Superintendent of Documents, Government Printing Office, Washington, D. C., at 5 cents each.

‡ Committee: Thomas Parran, *chairman*, H. H. Hazen, Washington, D. C., Arthur H. Sanford, Rochester, Minn., F. E. Seneear, Chicago, Ill., Walter M. Simpson, Dayton, Ohio, R. A. Vonderlehr, Assistant Surgeon General United States Public Health Service.



not more than two tests which they desired to perform for purposes of evaluation.

The number of laboratory directors requesting an invitation to participate in this work was greater than could be included because of the limitation of funds. Thirty-nine directors of State, municipal, or private laboratories expressed a desire to be included in the study. Of these, 30 were selected because of the priority of their requests or because of their strategic position in furthering the program for the control of syphilis in this country. The serologists in 11 State laboratories, 5 municipal laboratories, and 14 private laboratories were extended invitations. Of these, 9 specified the desire to perform only one method and 21 requested that their performance of two methods be evaluated. Thus, 51 performances of 19 separate serodiagnostic methods were available for evaluation. The serodiagnostic procedures and the frequency with which they were performed were as follows: Eagle flocculation, 1 laboratory; Hinton flocculation, 1 laboratory; Johns flocculation, 1 laboratory; Kahn presumptive flocculation, 2 laboratories; Kahn standard flocculation, 12 laboratories; Kline diagnostic flocculation, 7 laboratories; Kline exclusion flocculation, 2 laboratories, and Kolmer complement fixation, 14 laboratories. The originator of each of these serologic methods performed an examination on a comparable sample of blood in order that the study might be properly controlled.

The remaining eleven performances comprised a heterogeneous group of serologic tests which had not been given consideration in the first study to evaluate serodiagnostic tests for syphilis. These included the following methods: Bellevue Hospital complement fixation, 1 laboratory; Eagle complement fixation, 1 laboratory; Meinicke clarification reaction, 1 laboratory; Micro-Hinton flocculation, 1 laboratory; New York State complement fixation, 1 laboratory; modified Rosenthal micro-flocculation, 1 laboratory, and other modifications of the complement fixation method, 5 laboratories. With the exception of the Eagle complement fixation and the Micro-Hinton flocculation tests no control examinations were made for comparison with the eleven methods in the heterogeneous group.

The procedure followed in the collection and transportation of specimens was the same as that which was described for the original study,<sup>2</sup> except that the groups and numbers of donors were more limited. Blood specimens were selected from only two groups—approximately 200 specimens were from known syphilitic donors and approximately 100 from normal presumably non-syphilitic donors. Each group of syphilitic donors included about 10 with untreated secondary syphilis and approximately 190 syphilitic patients who had received varying amounts of treatment. A total of approximately 300 blood specimens was, therefore, submitted to each of the participating serologists in the State and local laboratories. Comparable samples were submitted simultaneously to those performing the control tests as described above. Thus, a total of 18,840 samples was distributed to the 39 serologists performing the 19 tests.

Because of the practical difficulties encountered in distributing comparable samples to all participants at the same time, it was found necessary to establish four separate groups of serologists. The serologists in each group received comparable samples from the same series of donors. Thus, in the tables and figures which follow, it is possible only to evaluate the performance of any given test with regard to its sensitivity and specificity as compared with other performances from the same series of donors. It is, therefore, obvious that no comparisons can be drawn between the percentages of sensitivity and specificity of the methods employed in the four different series as shown in the tables and graphs, or between those found in the results of the first evaluation study.<sup>1</sup>

The specimens for the evaluation of a given test were taken in such quantity as to provide a comparable sample for all laboratories performing that test. Whole blood specimens were collected in dry sterile glass syringes under aseptic conditions, transferred to uniform glass tubes, and stoppered with sterile corks. All laboratories performing two tests were requested to perform only one test on a single sample. An additional sample from a different series was furnished for the second test and each was designated by a code letter in order that there might be no confusion in identification.

The donors were carefully chosen, and accurate records were made of the pertinent facts in the history and physical examination. Reasonable care was taken to insure that donors would be available for clinical reexamination in the event that discrepancies existed in the reports of the participants. When more than half of the serologic reports on a specimen of blood from a patient suspected of having syphilis were negative, the patient, with a few exceptions, was subjected to a thorough clinical reexamination and to any indicated special examinations. The donors in the normal presumably nonsyphilitic group on whom more than one positive or more than two doubtful results were reported were serologically reexamined by the participating serologists and given a clinical reexamination by one or more syphilologists. Only the original serologic report was considered in computing the percentage of negative reports.

The Committee has found the evaluation of doubtful reports impracticable. A logical method is lacking for determining the amount of credit to be assigned or the deduction to be made in respect to such reports. There was no general agreement in the proposals offered by the participating serologists in the first study for the evaluation of doubtful reports. Throughout this study specimens giving doubtful reactions are included in the columns headed "specimens examined" but are not counted as positive or partially positive reports in determining percentages of positive reports or percentages of negative reports. Although in this study the doubtful reports have been given a negative rating, the Committee recognizes that in clinical practice a doubtful report may often be of value.

The Committee decided that in the publication of the results obtained by the participating serologists in State and local laboratories the names of the serologists and laboratories would not be published but would be designated by an arbitrarily chosen number. The director of each laboratory, however, has been furnished with the clinical diagnosis of each donor and with the results of the control serologic test compared with the results obtained in his laboratory. He has also been provided with the results obtained by other unidentified serologists who tested com-

parable specimens in the same series. Thus, the laboratory director is able not only to compare the results of the serologic testing in his laboratory with the control performance, but also to correlate the serologic findings with the clinical diagnoses.

TABLE 1

SENSITIVITY OF SERODIAGNOSTIC TESTS FOR SYPHILIS IN SERIES I (KOLMER COMPLEMENT FIXATION TEST) BASED UPON THEIR ABILITY TO DETECT SYPHILIS IN BLOOD SPECIMENS FROM 10 CASES OF UNTREATED SECONDARY SYPHILIS AND 190 CASES OF SYPHILIS WITH VARYING AMOUNTS OF TREATMENT, AND THE SPECIFICITY OF SERODIAGNOSTIC TESTS FOR SYPHILIS IN SERIES I BASED UPON THEIR ABILITY TO EXCLUDE SYPHILIS IN 100 BLOOD SPECIMENS FOR NORMAL PRESUMABLY NON-SYPHILITIC INDIVIDUALS

PARTICIPATING LABORATORIES	SENSITIVITY					SPECIFICITY				
	Total patients with syphilis (200)					Normal presumably non-syphilitic individuals (100)				
	Specimens examined	Doubtful reports	Positive reports	Percentage of positive reports	Specimens hemolyzed or physiologically damaged	Anticomplementary specimens	Specimens examined	Doubtful reports	False positive reports	Percentage of false positive reports
Kolmer complement fixation test:										
Control*.....	200	1	118	59.0			100			100.00
No. 1.....	200	8	108	54.0			100	7	3	3.00
No. 2.....	199		95	47.7	1		100		2	2.00
No. 3.....	199	4	121	60.8	1		99		3	3.03
No. 4.....	199	8	114	57.3	1	5	99	3		100.00
No. 5.....	199	1	142	71.4	1	13	99	2		100.00
No. 6.....	198	4	63	31.8	2	1	100			100.00
No. 7.....	193	11	119	61.7	7	3	99	2		100.00
No. 8.....	200	1	123	61.5			99		1	1.01
No. 9.....	183	2	92	50.3	17	3	90	1	1	1.11
No. 10.....	198	15	80	40.4	2		100	1		100.00
No. 11.....	199	7	84	42.2	1	1	100		1	1.00
No. 12.....	192		116	60.4	8	3	99			100.00
No. 13.....	190	23	67	35.3	10		98			100.00
No. 14.....	199	8	105	52.8	1	1	95	1		100.00

\* Performed by John A. Kolmer, Philadelphia, Pa.

#### EVALUATION OF SENSITIVITY

The results obtained from the serologic testing of blood specimens from syphilitic donors permit a determination of the degree

TABLE 2

SENSITIVITY OF SERODIAGNOSTIC TESTS FOR SYPHILIS IN SERIES II (EAGLE COMPLEMENT FIXATION TEST, HINTON TEST, KLINE DIAGNOSTIC TEST, MODIFIED COMPLEMENT FIXATION TEST, AND NEW YORK STATE COMPLEMENT FIXATION TEST) BASED UPON THEIR ABILITY TO DETECT SYPHILIS IN BLOOD SPECIMENS FROM 12 CASES OF UNTREATED SECONDARY SYPHILIS AND 201 CASES OF SYPHILIS WITH VARYING AMOUNTS OF TREATMENT, AND THE SPECIFICITY OF SERODIAGNOSTIC TESTS FOR SYPHILIS IN SERIES II BASED UPON THEIR ABILITY TO EXCLUDE SYPHILIS IN 103 BLOOD SPECIMENS FOR NORMAL PRESUMABLY NON-SYPHILITIC INDIVIDUALS

TEST PERFORMED AND PARTICIPATING LABORATORIES	SENSITIVITY						SPECIFICITY						
	Total patients with syphilis (213)						Normal presumably non-syphilitic individuals (103)						
	Specimens exam- ined	Doubtful reports	Positive reports	Percentage of posi- tive reports	Specimens hemo- lyzed or phys- ically damaged	Anticomplemen- tary specimens	Specimens exam- ined	Doubtful reports	False positive re- ports	Percentage of false positive reports	Percentage of neg- ative reports	Specimens hemo- lyzed or phys- ically damaged	Anticomplemen- tary specimens
Eagle complement fixa- tion test:													
Control*.....	204	8	171	83.8	9	1	102				100.00	1	
No. 1.....	197		163	82.7	16	1	102				100.00	1	
Hinton test:													
Control†.....	197	5	175	88.8	16		100				100.00	3	
No. 2.....	207	2	181	87.4	6		103	1			100.00		
No. 3‡.....	211	2	194	91.9	2		100				100.00	3	
Kline diagnostic test:													
Control§.....	205	10	182	88.8	8		103	1	1	0.97	99.03		
No. 4.....	209	1	145	69.4	4		103	1			100.00		
No. 5.....	208	18	178	85.6	5		90	2	1	1.11	98.89	13	
No. 6.....	205	2	190	92.7	8		103		2	1.94	98.06		
No. 7.....	209	22	168	80.4	4		103	1			100.00		
No. 8.....	207	5	193	93.2	6		103	1	4	3.88	96.12		
No. 9.....	208	17	177	83.1	5		103	2	1	0.97	99.03		
No. 10.....	207	11	185	89.4	6		102	2			100.00	1	
Modified complement fixation test:													
No. 11.....	211	6	169	80.1	2		102	6	2	1.96	98.04	1	1
New York State comple- ment fixation test:													
No. 12¶.....	207	15	145	70.0	6	1	102	2			100.00	1	

\* Performed by Harry Eagle, Philadelphia, Pa.

† Performed by Wm. A. Hinton, Boston, Mass.

‡ Performed the Micro-Hinton test.

§ Performed by B. S. Kline, Cleveland, Ohio.

¶ Not performed in New York State Department of Health Laboratories.

TABLE 3

SENSITIVITY OF SERODIAGNOSTIC TESTS FOR SYPHILIS IN SERIES III (BELLEVUE HOSPITAL COMPLEMENT FIXATION TEST, EAGLE FLOCCULATION TEST, KAHN PRESUMPTIVE TEST, KLINE EXCLUSION TEST, MEINICKE MICRO-FLOCCULATION TEST, FOUR MODIFIED COMPLEMENT FIXATION TESTS, AND MODIFIED ROSENTHAL FLOCCULATION TEST) BASED UPON THEIR ABILITY TO DETECT SYPHILIS IN BLOOD SPECIMENS FROM 13 CASES OF UNTREATED SECONDARY SYPHILIS AND 173 CASES OF SYPHILIS WITH VARYING AMOUNTS OF TREATMENT, AND THE SPECIFICITY OF SERODIAGNOSTIC TESTS FOR SYPHILIS IN SERIES III BASED UPON THEIR ABILITY TO EXCLUDE SYPHILIS IN 101 BLOOD SPECIMENS FOR NORMAL PRESUMABLY NON-SYPHILITIC INDIVIDUALS

TEST PERFORMED AND PARTICIPATING LABORATORIES	SENSITIVITY						SPECIFICITY						
	Specimens exam- ined	Doubtful reports	Positive reports	Percentage of posi- tive reports	Specimens hemo- lyzed or physi- cally damaged	Anticomplemen- tary specimens	Normal presumably non-syphilitic individuals (101)						
							Specimens exam- ined	Doubtful reports	False positive re- ports	Percentage of false positive reports	Percentage of neg- ative reports	Specimens hemo- lyzed or physi- cally damaged	Anticomplemen- tary specimens
Bellevue Hospital comple- ment fixation test: No. 1*.....	184	8	135	73.4	2	14	100	20	1	1.00	99.00	1	9
Eagle flocculation tests: Control†.....	185		144	77.8	1		99				100.00	2	
No. 2.....	186	6	143	76.9			100				100.00	1	
Kahn presumptive test: Control‡.....	186	5	148	79.6			98		1	1.02	98.98	3	
No. 3.....	175	10	141	80.6	11		92	6	1	1.09	98.91	9	
No. 4.....	184	2	140	76.1	2		100		1	1.00	99.00	1	
Kline exclusion test: Control§.....	184	14	156	84.8	2		101	4			100.00		
No. 5.....	186	7	170	91.4			101	32	8	7.92	92.08		
No. 6.....	185	7	161	87.0	1		99	3	3	3.03	96.97	2	
Meinicke micro-floccu- lation test: No. 7.....	182	24	94	51.6	4		99	1			100.00	2	

\* Not performed in the laboratory of the Bellevue Hospital.

† Performed by Harry Eagle, Philadelphia, Pa.

‡ Performed by M. B. Kurtz, Lansing, Mich.

§ Performed by Charles R. Rein, New York, N. Y.



TABLE 3—*Concluded*

TEST PERFORMED AND PARTICIPATING LABORATORIES	SENSITIVITY						SPECIFICITY						
	Total patients with syphilis (186)						Normal presumably non-syphilitic individuals (101)						
	Specimens exam- ined	Doubtful reports	Positive reports	Percentage of posi- tive reports	Specimens hemo- lyzed or physi- cally damaged	Anticomplemen- tary specimens	Specimens exam- ined	Doubtful reports	False positive re- ports	Percentage of false positive reports	Percentage of neg- ative reports	Specimens hemo- lyzed or physi- cally damaged	Anticomplemen- tary specimens
Modified complement fixation tests:													
No. 8.....	179	12	93	52.0	7	1	100		1	1.00	99.00	1	
No. 9.....	182	11	85	46.7	4	4	100				100.00	1	
No. 10.....	183	2	149	81.4	3		100	4	9	9.00	91.00	1	
No. 11.....	186	46	69	37.1			99	1			100.00	2	
Modified Rosenthal flocculation test:													
No. 12.....	179	22	61	34.1	7		100	8	1	1.00	99.00	1	

of sensitivity of the various serologic procedures in the same series. The percentage of positive reports on the different tests performed by each of the fifteen serologists in Series I to IV will be found in tables and figures 1 to 4. The percentage of positive reports was obtained by dividing the number of positive reports by the total number of samples examined. The samples examined by each serologist represented the total number submitted in each series of syphilitic donors less the number reported as hemolyzed, as physically damaged, or as not received.

#### EVALUATION OF SPECIFICITY

The specificity of the various serologic tests has been determined from the results obtained by the serologic testing of blood specimens from normal presumably nonsyphilitic individuals. All of these donors were included in a selected group in which the prevalence of syphilis was believed to be lower than the average for the whole population. The group consisted almost entirely of students and members of the staffs of medical schools and hospitals. The total number of samples examined, the number

TABLE 4

SENSITIVITY OF SERODIAGNOSTIC TESTS FOR SYPHILIS IN SERIES IV (JOHNS FLOCCULATION TEST AND KAHN STANDARD TEST) BASED UPON THEIR ABILITY TO DETECT SYPHILIS IN BLOOD SPECIMENS FROM 10 CASES OF UNTREATED SECONDARY SYPHILIS AND 192 CASES OF SYPHILIS WITH VARYING AMOUNTS OF TREATMENT, AND THE SPECIFICITY OF SERODIAGNOSTIC TESTS FOR SYPHILIS IN SERIES IV BASED UPON THEIR ABILITY TO EXCLUDE SYPHILIS IN 100 BLOOD SPECIMENS FOR NORMAL PRESUMABLY NON-SYPHILITIC INDIVIDUALS

TEST PERFORMED AND PARTICIPATING LABORATORIES	SENSITIVITY						SPECIFICITY						
	Specimens examined	Total patients with syphilis (202)					Normal presumably non-syphilitic individuals (100)						
		Doubtful reports	Positive reports	Percentage of positive reports	Specimens hemolyzed or physically damaged	Anticomplementary specimens	Specimens examined	Doubtful reports	False positive reports	Percentage of false positive reports	Percentage of negative reports	Specimens hemolyzed or physically damaged	Anticomplementary specimens
Johns flocculation test:													
Control*.....	197		119	60.4	5		99		1	1.01	98.99	1	
No. 1.....	198	16	81	40.9	4		94	1	1	1.06	98.94	6	
Kahn standard diagnostic test:													
Control†.....	202	3	153	75.7			99	1			100.00	1	
No. 2.....	199	11	131	65.8	3		100	4	2	2.00	98.00		
No. 3.....	189	22	71	37.6	13		99	1			100.00	1	
No. 4.....	196	2	156	79.6	6		97	3	1	1.03	98.97	3	
No. 5.....	200	13	141	70.5	2		100				100.00		
No. 6.....	197	11	139	70.6	5		98	1			100.00	2	
No. 7.....	195	18	146	74.9	7		96	1	2	2.08	97.92	4	
No. 8.....	200	9	143	71.5	2		100	2			100.00		
No. 9.....	198	4	134	67.7	4		100				100.00		
No. 10.....	196		145	74.0	6		100				100.00		
No. 11.....	194	14	113	58.2	8		94				100.00	6	
No. 12.....	200	6	157	78.5	2		100	2			100.00		
No. 13.....	202	7	169	83.7			100		1	1.00	99.00		

\* Performed by F. M. Johns, New Orleans, La.

† Performed by Reuben L. Kahn, Ann Arbor, Mich.

of false positive reactions reported by the participants, the percentage of false positive reports, and the percentage of negative reports are also given in tables and figures 1 to 4.



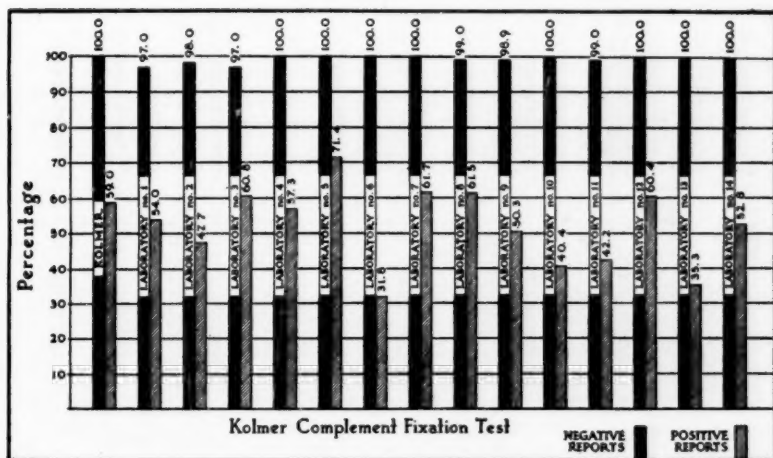


FIG. 1. SENSITIVITY OF BLOOD TESTS IN SERIES I

Based on the percentage of positive reports in a group of 200 patients with syphilis contrasted with the specificity of blood tests based on the percentage of negative reports in a group of 100 normal presumably non-syphilitic individuals.

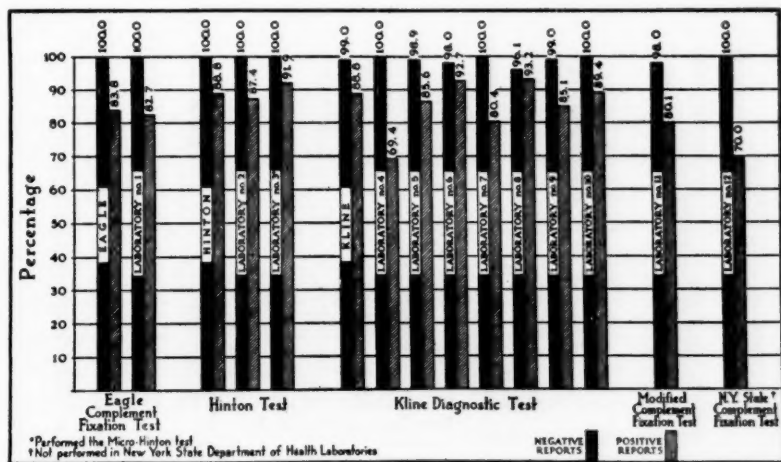


FIG. 2. SENSITIVITY OF BLOOD TESTS IN SERIES II

Based on the percentage of positive reports in a group of 213 patients with syphilis contrasted with the specificity of blood tests based on the percentage of negative reports in a group of 103 normal presumably non-syphilitic individuals.

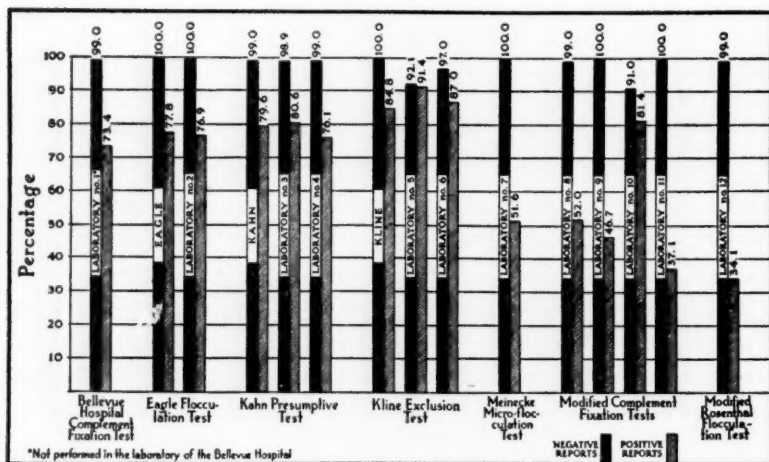


FIG. 3. SENSITIVITY OF BLOOD TESTS IN SERIES III

Based on the percentage of positive reports in a group of 186 patients with syphilis contrasted with the specificity of blood tests based on the percentage of negative reports in a group of 101 normal presumably non-syphilitic individuals.

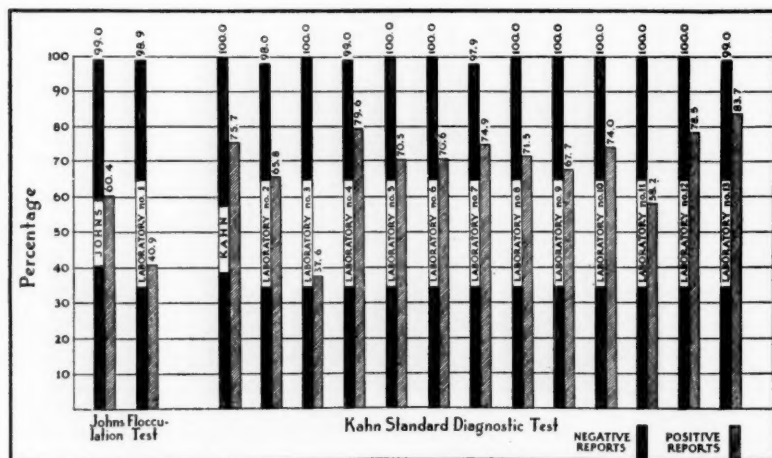


FIG. 4. SENSITIVITY OF BLOOD TESTS IN SERIES IV

Based on the percentage of positive reports in a group of 202 patients with syphilis contrasted with the specificity of blood tests based on the percentage of negative reports in a group of 100 normal presumably non-syphilitic individuals.

## DISCUSSION AND RECOMMENDATIONS

In this undertaking the Committee has attempted to meet, more closely than was possible in the first evaluation project, the conditions encountered in ordinary practice. A study of the tables reveals that in some of the State and local laboratories the serologic testing does not compare favorably with the results achieved in the laboratories of the originators of the methods. On the other hand, it is pleasing to note that in other State and local laboratories the results achieved are at least comparable to those obtained with the control tests. Particular attention should be directed to the relative uniformity of the results obtained in practically all of the laboratories in the performance of certain tests. A test of equal efficiency from the standpoint of sensitivity and specificity which yields uniformly successful results in the hands of practically all serologists, is distinctly superior to one which yields less uniform results.

It is quite apparent that the performance of some of the tests in some of the laboratories is inadequate. Obviously, in certain laboratories improvement should be brought about in the performance of well recognized tests, the value of which has been demonstrated in this study and in the first evaluation project. Likewise, some of the tests should either be modified to increase their sensitivity or specificity or both, or be abandoned.

An efficient serodiagnostic test for syphilis should possess specificity of 100 per cent. Any test which yields even one per cent of false positive reactions should be so modified as to increase its specificity, even with some slight sacrifice of sensitivity.

The examination of the clinical records of the presumably syphilitic donors in this study again emphasizes the fact that a serologic diagnosis of syphilis unsupported by history or clinical evidence should never be made on the basis of a single positive blood reaction. The Committee found no justification for a diagnosis of syphilis in a number of cases, particularly in one series, in which the diagnosis had been based upon a single false positive reaction. The reports of the serologic testing of the blood specimens from these donors were not included in the evaluation. If a positive blood test is obtained in a person who

presents no history or clinical evidence of syphilis, the test should be repeated in the same laboratory, or in another laboratory, utilizing two or more different tests.

The studies made by this Committee show that if two tests are to be performed, it is immaterial whether two efficient complement fixation tests, two efficient flocculation tests, or a combination of one efficient flocculation test and one efficient complement fixation test is selected. As in the first evaluation project this study again indicates relatively equal value to the clinician of efficient complement fixation tests and efficient flocculation tests as applied to blood specimens.

The experience of the Committee shows that it is satisfactory to report the results of qualitative tests as merely positive, doubtful or negative. In this way the confusion arising from the use of various symbols is avoided.

The directors of laboratories performing serodiagnostic tests for syphilis should have, and should avail themselves of, the opportunity of comparing their results with those of well qualified serologists in other laboratories performing the same test on comparable samples from known syphilitic and presumably non-syphilitic individuals. The Committee recommends that such a system of comparative examination of tests be extended annually to all State laboratories. In turn, the State laboratories should offer a similar opportunity to local laboratories.

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- (2) Evaluation of serodiagnostic tests for syphilis. Ven. Dis. Inform., Washington, December 1934, **15**: 387; also J. A. M. A., Chicago, December 1, 1934, **103**: 1705.

## A METHOD FOR DETERMINING THE OPTIMUM DOSE AND SENSITIVITY OF ANTIGENS USED IN THE WASSERMANN TEST\*

FREI<sup>†</sup>, BOERNER AND MARGUERITE LUKENS

*From the Laboratories of the Graduate Hospital, University of Pennsylvania*

The methods for antigenic titration usually recommended by the authors of the various modifications of the complement-fixation test for syphilis are very similar in principle. The smallest amount of antigen that will give complete fixation with a constant dose of positive serum is called a unit and this is then used as a criterion for determining the dose of antigen to be employed in the test. It is obvious that an antigenic unit thus obtained is not standard because of the fact that positive sera vary in their antibody content, although many serologists have sought to overcome this difficulty by using a mixture of four or more syphilitic sera.

After obtaining the antigenic unit by titrating with positive serum, the optimum dose to be employed has been empirically determined to be somewhere between the antigenic unit and one-tenth of the anticomplementary dose. Kolmer<sup>1</sup> recommends ten units as a dose of his antigen. Recently he<sup>2</sup> has described a new method for preparing his antigen by reinforcing with acetone insoluble lipoids, which was found to increase antigenic sensitiveness with none or but slight increase of anticomplementary activity. Under these conditions, Kolmer recommended the use of 20 antigenic units, as this dose was still 40 to 60 times less than the anticomplementary unit and giving, therefore, a wide range of safety. Eagle<sup>3</sup> states that "the ordinary method of antigen titration which involves the determination of the antigen unit, is wholly inadequate" and recommends a method for titrating his antigen which is very similar to the one we are presenting. It is

\* Received for publication August 3, 1936.

certainly clear and acknowledged by nearly all serologists that the unit system is not very satisfactory due to the variability of positive sera.

After a careful study of this problem, we have used a method for determining the optimum dose and sensitivity of Wassermann antigens which appears to be an improvement due to its reproducibility with positive sera irrespective of their strength.

#### METHOD FOR DETERMINING THE OPTIMUM DOSE AND SENSITIVITY OF WASSERMANN ANTIGENS

The method for titrating antigens developed in this study includes two variables, i.e., the serum and the antigen. We be-

TABLE 1

SERUM	ANTIGEN DILUTIONS						
	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
cc.							
0.0125	—	—	—	2	4	3	±
0.025	—	—	3	4	4	4	±
0.05	±	4	4	4	4	4	1
0.1	3	4	4	4	4	4	2
0.2	4	4	4	4	4	4	2

The antigen used in this test was prepared according to Kolmer's new method.

lieve the method to be applicable to all complement-fixation tests. However, we have only used it in two modifications of the Wassermann test, namely, the Kolmer and the Eagle. The set-up of the test is the same as has been recommended except, in the place of one dose of serum several are employed.

Table 1 shows an antigen titration using the Kolmer method. This set-up can be modified depending upon the expectant range of the antigen and the strength of the positive serum employed. When the optimum range falls somewhere between the dilutions of 1:160 and 1:1280, as in the case of the Kolmer antigen, it may only be necessary to employ five or six dilutions of antigen. In the Eagle test the higher dilutions can be omitted as the range invariably falls in stronger dilutions; when a weakly positive serum is used the smaller doses of serum may be omitted.



REPRESENTATIVE DATA SHOWING THE MAXIMUM RANGE OF  
SENSITIVITY AND OPTIMUM DOSE OF KOLMER AND EAGLE  
ANTIGENS

The results obtained in the following titrations are presented in graph form. The curves represent the smallest amount of serum giving complete fixation with the various doses of antigen.

Chart I shows the results obtained when the same antigen was

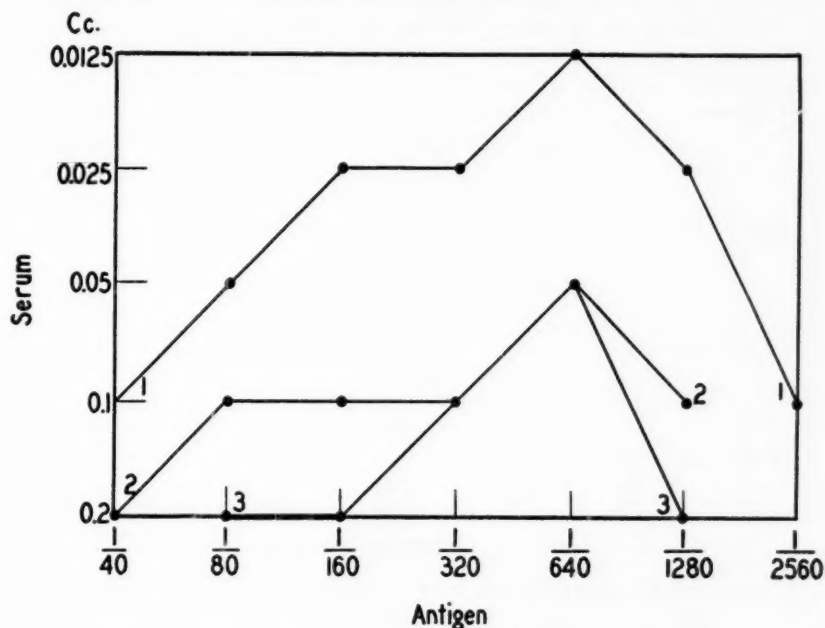


CHART I. KOLMER ANTIGEN, NEW METHOD, PREPARED BY KOLMER

No. 1 test, 1/3/36; No. 2 test, 2/14/36; No. 3 test, 1/10/36

tested on three different occasions employing a serum of different source and strength in each test. The range of maximum fixation was quite close and the optimum dose the same, irrespective of the strength of the serum used. The antigen used in these titrations was supplied by Dr. Kolmer.

Chart II shows the results obtained with an antigen prepared at the Graduate Hospital using Kolmer's new method. This antigen was tested on three different occasions using a serum of



different source and strength in each test. If we assume that the optimum dose should be about the middle of the maximum range, the first test gives the dose as  $1/320$ ; the second test between  $1/320$  and  $1/640$ ; the third test as  $1/640$ .

Chart III shows the optimum range obtained with nine Kolmer antigens, seven of which were prepared according to his new method, and two by the old method. It is interesting to note how close the optimum dose appears, irrespective of the strength of serum used for titrating or the method of preparation. The

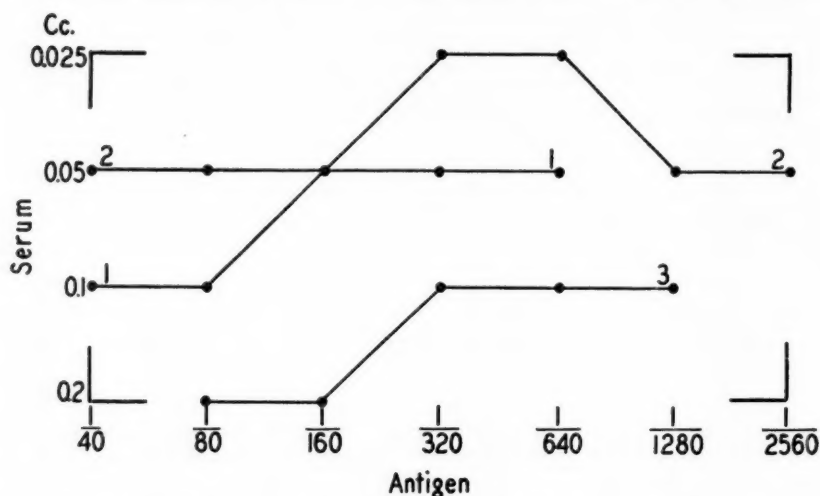


CHART II. KOLMER ANTIGEN, NEW METHOD, PREPARED AT GRADUATE HOSPITAL

No. 1 test, 1/10/36; No. 2 test, 1/15/36; No. 3 test, 1/21/36

optimum dose for five of these antigens was about  $1/320$ ; for three, between  $1/320$  and  $1/640$ , and one at  $1/640$ . When the optimum dose falls between two dilutions it appears safer to favor the stronger dilution, because of the fact that there is very often a precipitous drop in the fixability in the higher dilutions.

Chart IV shows the results obtained with two Eagle antigens which were prepared by Dr. Eagle and tested at different times. The Eagle modification<sup>5</sup> of the complement-fixation test was used. The results obtained indicate the optimum dose of anti-

gen 1 to be 1:80, and antigen 2 between 1:80 and 1:120. Eagle recommends a dose of about 1:100, which falls within the range of maximum sensitivity as determined by these tests.

In chart V, the upper three lines represent the results obtained with two Kolmer antigens prepared by his new method, and one

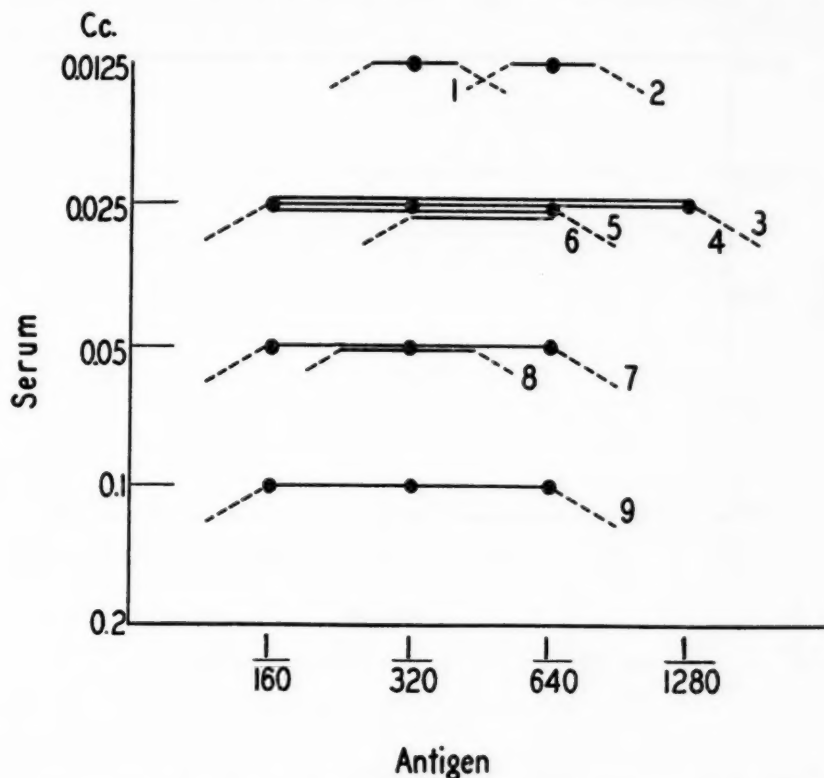


CHART III. Nos. 1, 4, 5, 6, 7 and 8 Kolmer antigens (new method) prepared at the Graduate Hospital. No. 2 antigen supplied by Dr. Kolmer (new method). Nos. 3 and 9. Kolmer antigens (old method) prepared Graduate Hospital.

antigen by his old method. The three antigens were tested at the same time with the same positive serum. The purpose of these tests was to compare the sensitivity of the new and old Kolmer antigens. The two lower lines shows the results with two other antigens, one prepared by the new method and the other

by the old. In this test a weaker positive serum was used. The results show a slight difference in sensitivity, as two of the antigens prepared by the new method give higher reading at one dilution as shown by the upper three lines in the chart. The two other antigens did not show any difference.

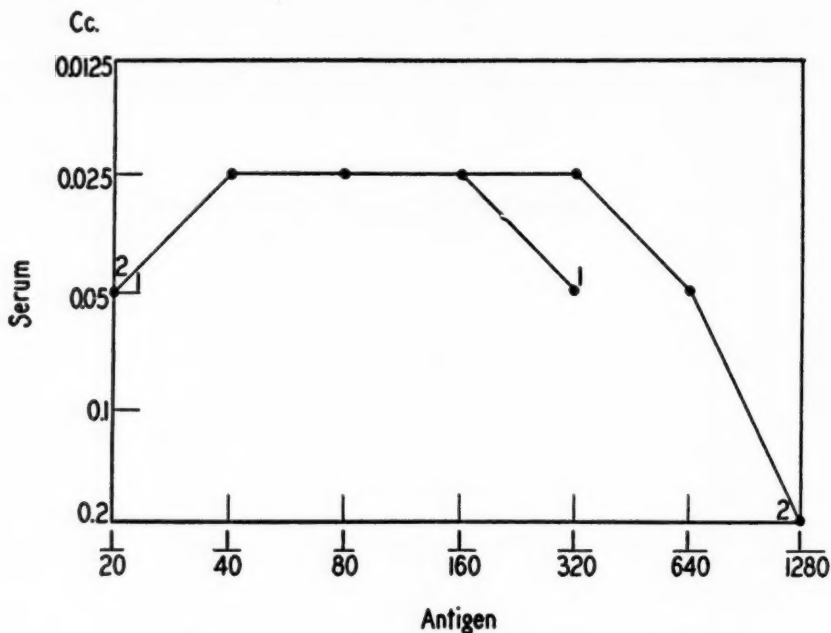


CHART IV. EAGLE ANTIGENS PREPARED BY EAGLE

No. 1 tested 1/27/36; No. 2 tested 2/3/36

#### DISCUSSION

As previously stated, the common practice of titrating Wassermann antigens with a single dose of strongly positive serum and a constant dose of complement, is not reproducible because of the variability of positive sera. From the experimental work presented, it is obvious that the sensitivity of an antigen can not be determined by this method. The method which we have presented includes two variables, the antigen and the positive serum. Titrations carried out in this manner give definite information

regarding the range of maximum fixation and the optimum dose should be somewhere near the middle of this range. The range of maximum fixation is not influenced by the strength of the positive serum used. Any positive serum giving fixation in a dose of 0.1 cc. or less can be used in this method, therefore the difficulty of obtaining suitable positive serum for titrating antigens is obviated. It is shown in chart I that the optimum dose was

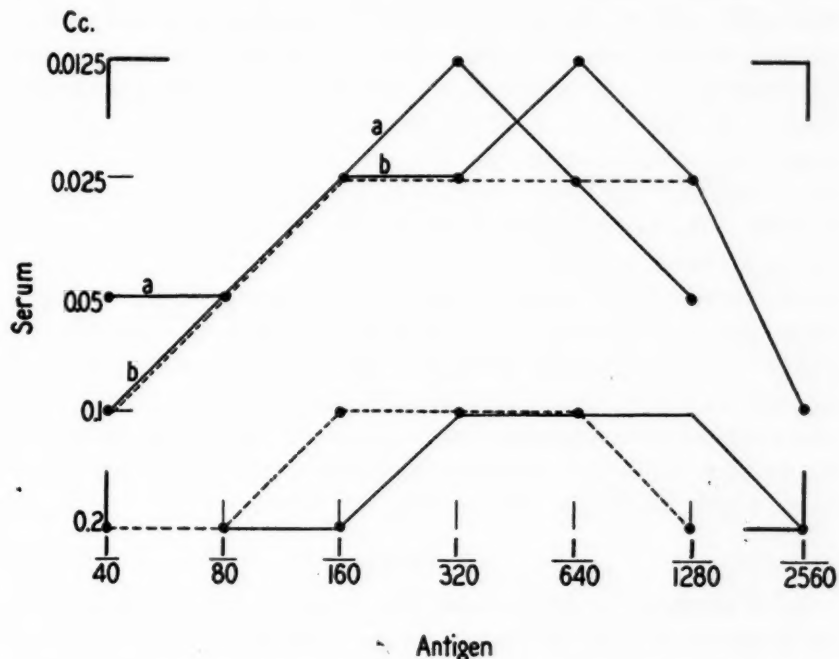


CHART V. Solid lines indicate Kolmer's new antigens. Broken lines indicate Kolmer's old antigen.

about the same when the antigen was tested at different times with sera of different strengths. Charts II, III and IV show that the range of maximum fixation of antigens prepared by the same method are very close. The Kolmer antigen prepared either by his old or new method has about the same range of maximum fixation, which in most cases lay between a dilution of 1:160 and 1:1280 (see chart V). From our experiments it would appear

that a dose between 1:320 and 1:640 would be proper for either antigen.

There is no doubt that many serologists working with the unit system have been using antigens outside of their range of maximum fixation. This has certainly been the case in our own laboratories. This was undoubtedly due to the fact that when antigens were tested with strongly positive sera, the antigenic unit fell in the higher dilutions and it was difficult to determine the exact unit due to the differences in positive sera, even when pooled positive sera was used. It is of interest to note from the experiments here presented that there seems to be no exact correlation between the unit of antigen as usually obtained and its sensitivity. An antigen may give an antigenic unit in a dilution of 1:2560 and not be as sensitive as another that gives a unit of 1:1280. We have compared the Kolmer and the Eagle antigens under identical conditions and have found that the Eagle antigen would not fix in the higher dilutions in which the Kolmer antigen was capable of fixing, but was more sensitive in its optimum range than the Kolmer antigen. Eagle recommends a dose of antigen of 1:120, which dose according to our tests falls within the range of the optimum dose. Eagle<sup>3</sup> decided upon this dose by using a method of titration very similar to the one presented here, which, no doubt, accounts for our agreement.

#### CONCLUSION

1. A method for determining the range of maximum fixation of antigens used in the Wassermann reaction is described.
2. This method offers a better means for determining the optimum dose of antigen to be used in the Wassermann test.
3. The value of this method for studying the relative sensitivity of antigens is discussed and experimental data given.

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## THE UNIVERSAL ELECTRO SCOPOMETER\*

WILLIAM G. EXTON

*From the Laboratory and Longevity Service of The Prudential Insurance Company,  
Newark, New Jersey*

The original design of the Photo-Electric Scopometer<sup>1,2</sup> included several objectives: a sensitive but fool-proof instrument that would measure transmitted or absorbed light without having to change or modify methods in any way to fit the instrument; a sturdy instrument that people without training in mathematics or electricians could operate quickly and easily; and, above all, an instrument that would attain the utmost precision. The achievement of these objectives has been abundantly confirmed by an experience of seven years with a number of Scopometers in many different hands, in our own and other laboratories, and comparisons of the voluminous data thus acquired with the published data of others indicate the superior precision of the Scopometer.

While this experience has amply demonstrated the many practical advantages of measurement with pre-determined calibrations, it has also taught that a direct measurement, like Duboscq photometry, is sometimes better and sometimes indispensable. I have, therefore, re-designed the Scopometer to provide a direct measurement with comparison standards in addition to its original measurement with preliminary calibrations. With this provision for both methods of measurement in one instrument the Scopometer fulfils every conceivable demand of the laboratory for such optical measurements because it is now unlimited in its applications to every method and contingency. The new instrument, or Universal Electro-Scopometer as it is called, therefore supersedes the older.

\* Read before the Fifteenth Annual Convention of the American Society of Clinical Pathologists, Kansas City, Missouri, May 8 to 10, 1936. Received for publication, September 4th, 1936.



## DESCRIPTION OF THE ELECTRO-SCOPOMETER

From the illustrations (figs. 1 and 2) it will be seen that the Scopometer consists of a bench supporting a balanced optical axis with housings for a photo-electric tube on each end and a light source in the middle. Recesses for specimen cups, etc. are interposed in the axis symmetrically on both arms. Of the two small boxes on the bench between the housings, the one on the right with snap switch in front holds the power supply, and the one on the left with push-pull switch in front holds the Wheatstone Bridge which connects the photo-electric tubes with power supply and galvanometer. Terminals for galvanometer and power connections are on the back of the instrument. The only movable parts of the instrument are connected with the two dials seen in front of the Scopometer. Turning the dial next to the left photo-tube housing fixes the dimensions of a calibrated variable aperture. Turning the dial next to the lamp house fixes the position of the light source between the

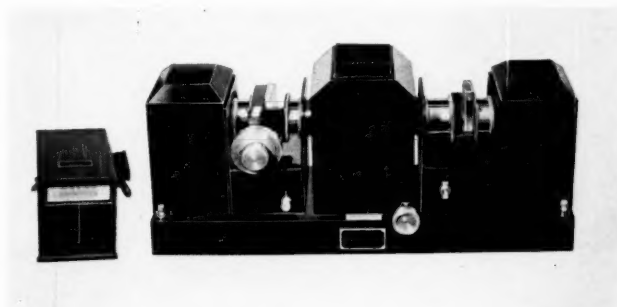


FIG. 1. THE UNIVERSAL ELECTRO-SCOPOMETER

two photo-electric tubes. The circuit diagram (fig. 3) shows how the electrical matches the perfect optical symmetry.

The photo-electric cell or tube is, of course, the heart of any photo-electric measuring device. Of those I have experimented with, or whose characteristics have been published, the only ones free from inconstancies which affect precision photometry are well-made specially processed cells of the vacuum photo emissive type. While any other cells might be used, the Scopometer is equipped with a pair of the caesium magnesium tubes (Westinghouse No. W L 770) developed by Dr. Harvey C. Rentschler, Director of Research, Westinghouse Lamp Company, Bloomfield, N. J. Some of these cells have been in continuous use in the Prudential laboratory seven and many four years, and none of them have so far shown any perceptible change or deterioration although repeatedly tested. Our experience thus supports theoretical expectations that the well made vacuum photo emissive tube is the only type of photo-electric cell now

available which is permanent in life and constant in function. Since these cells are free from fatigue, environmental and other inconstancies, their stability and exact functional reproducibility are reliable.

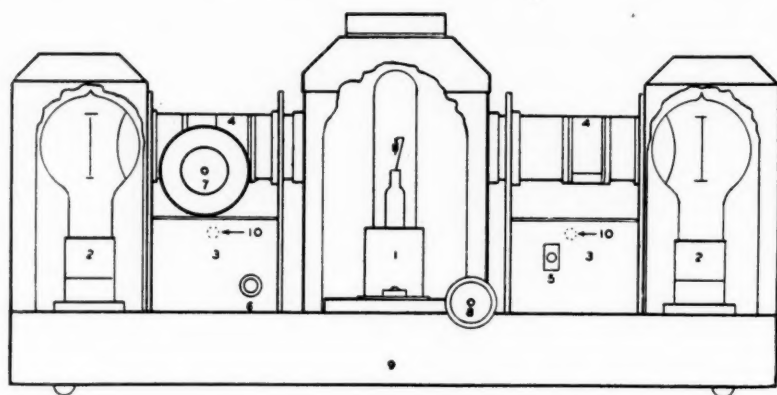


FIG. 2. DIAGRAM OF SCOPOMETER

1, lamp (light source); 2, photo tubes; 3, electric circuit housings; 4, specimen pockets; 5, power switch (lead on opposite side); 6, bridge circuit switch; 7, calibrated variable aperture; 8, lamp movement control; 9, base; 10, galvanometer leads on opposite side of circuit housings (3).

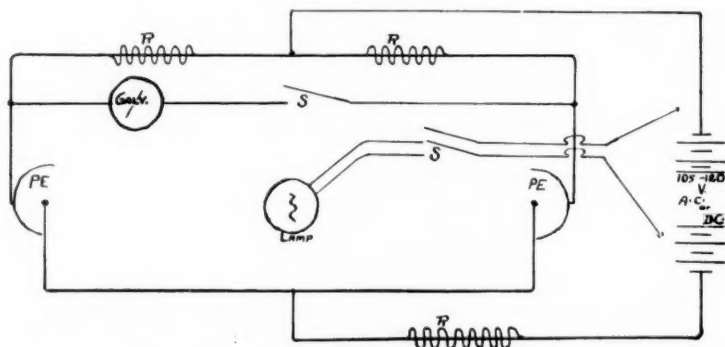


FIG. 3. ELECTRICAL CIRCUIT OF SCOPOMETER

Figure 4 shows the color sensitivity of Dr. Rentschler's cells. While the spectrum looks a little more blue to them than it does to the human eye, they are more than red-sensitive enough to measure efficiently hemoglobin and all of the other yellow, red, and brown solutions of clinical pathological interest

without filters of any kind. Dr. Rentschler's cells are so sensitive to turbidity that they measure the slightest by transmitted light better than nephelometers and tyndallmeters by reflected light. The photo tubes are housed in light, dust, and smoke-proof compartments, and when firmly in place need no care at all. In fact, they should not be handled because moisture from the hands may cause surface leaks which, however, can be cured by wiping and drying the surface with alcohol. It may also be noted here that the photo tubes in the Scopometer form the variable arms of a simple Wheatstone bridge and that this arrangement makes the Scopometer independent of fluctuations in line current.

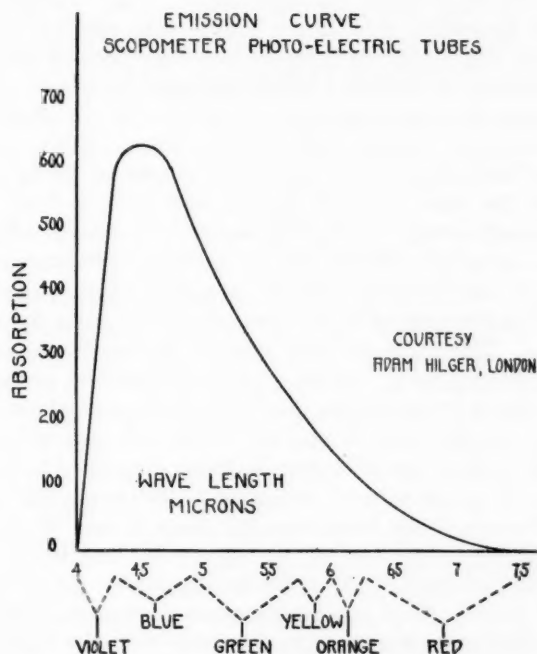


FIG. 4. EMISSION CURVE OF DR. RENTSCHLER'S PHOTO-ELECTRIC TUBES

The same arrangement fixes the rôle of the galvanometer which the Scopometer employs only as a null indicator of the equality of photo-electric currents. This in turn makes the Scopometer independent of deflections, sensitivity and other characteristics of galvanometers, and thereby allows a wide latitude in their choice. A Leeds and Northrup stock galvanometer No. 2420 C with three-second period and 0.025 microampere sensitivity is the usual equipment. They are durable, do not require levelling or protection from vibration under usual conditions, and are amply sensitive for clinical pathological requirements. If more sensitivity be desired, Messrs. Leeds and Northrup supply 0.001

microampere sensitive suspensions which fit in the stock 2420 galvanometer. They are very satisfactory. For the utmost sensitivity, it is well to choose a galvanometer having as short a period as possible, such as the Leeds and Northrup No. 2480 which has a period of only three seconds with a sensitivity of 0.0005 microampere.

There are no lenses, mirrors, or prisms in the optical system of the Scopometer. This departure from accustomed practise has the very practical advantage of eliminating all the troubles of cleaning glass surfaces which accumulate dirt and scum from laboratory smokes and vapors. In place of the usual lens system, a series of stops limits the light falling on the photo tubes to a cone blocked out by the stop nearest the light source. The usual light source is a 100 watt stock projection lamp of the T type with medium pre-focus base. Lamps operating with higher or lower wattage may be used for special purposes. The position of the light source between the photo tubes assures equality of illumination, provides perfect balancing of the photo tubes, and cancels changes in light intensity caused by fluctuations in line voltage.

The recesses for the specimen cups on both sides of the lamp house are spacious enough for cups of various sizes as well as light filters, etc. While light filters offer advantages which cannot well be dispensed with when they are needed, the use of light filters involves practical disadvantages which make it advisable to do without them when they are unnecessary. In the numerous applications of the Scopometer which have so far been made, light filters were never necessary. The glass specimen cups of the usual equipment hold a layer of liquid 6 mm. thick. Three cubic centimeters of sample suffice to fill them. Smaller or larger samples may be used with special cups.

The dial with attached pointer and scale next to the left photo tube house changes the size of the variable aperture in linear proportion to its area. The dial next to the lamp house moves the lamp in the optical axis and its scale indicates the distance of the lamp from the photo tubes. It also actuates a pointer which shows the position of the lamp. The combination of variable aperture and movable light source gives the Scopometer its unique flexibility because either may be used for measurement or compensation. Thus, in Calibration Scopometry measurements are made with the variable aperture and compensation with the lamp movement, while in Comparison Scopometry measurements are made with lamp movements and compensation with the variable aperture.

The Scopometer works on A.C. or D.C. and is ready for operation when plugged into any 105-130 volt light socket. From this description it is evident that both of its null methods of measurement are independent of line current fluctuations and the characteristics of photo tubes and galvanometers.

#### CALIBRATION SCOPOMETRY

Calibration Scopometry is a purely empirical method limited only by the reproducibility of materials it is desired to measure. The concentrations of

unknown samples of such materials are determined directly by referring their variable aperture scale readings to a pre-determined calibration of the scale reading-concentration equivalents of a material.

Preliminary to operation, i.e., all switches "off," light the galvanometer lamp and see that the galvanometer rests on "0" before snapping on the power switch which lights the Scopometer lamp, and throwing in the photo-electric circuit by pulling out the push pull switch. With this done operation begins at once by null-setting the system with clear water in both cups, or with the reagent if it has color or contains solvents. Specimen cups are best placed symmetrically nearest the photo tubes.

#### *To Null Set*

*With filled cups in place and variable aperture open (on "0"), balance the system by turning the lamp dial until the galvanometer rests on "0."*

#### *To Measure*

*Substitute unknown samples in the right cup and balance the system by turning the variable aperture dial until the galvanometer rests on "0."*

*When referred to an appropriate calibration, the scale readings at this point tell the concentrations of unknown samples directly.*

To calibrate the Scopometer for any method, prepare a series of standard samples in the same way one does for Duboscq colorimetry. Their number depends somewhat upon the desired range. In biochemistry the lowest concentration of interest corresponds with the low normal of a method and the highest concentration with the high pathological of a method. Such ranges, for example, run from 70 to 400 milligrams for blood sugar, from 25 to 100 milligrams for Non-Protein-Nitrogen, and from 0 to 100 milligrams for protein, etc. Between these limits it is better to have too many than too few intermediate concentrations. About ten in all usually suffice. Measure these standards in the Scopometer as directed and record the scale reading equivalent of grams, percentage, or any other desired unit for each concentration in graphic or tabular form. Graphs are conveniently made on cross section paper by plotting scale readings on the ordinate against concentrations on the abscissa and connecting the points. Such graphs should then be protected for permanent reference. Figure 5 shows calibrations made in this way for three different methods: blood sugar by picric acid<sup>13</sup>; blood creatinine by picric acid<sup>10</sup>, and urinary sugar by di-sodium-di-nitro-salicylate<sup>9</sup>.

The greatest accuracy will be secured by paying attention to the time element of methods or most favorable time for measuring a material. In this respect some methods have more latitude than others because some materials take more time than others to reach full intensity and become stable enough to measure, while other materials reach full intensity faster and must be measured promptly before they become unstable. Whatever its characteristics, the

time element of a material should be definitely known so that not only the calibration but all subsequent measurements are made in accordance with it. This point, so often ignored in practise, is essential to accuracy and applies to all measurements regardless of measuring device.

To determine the time element of a material, measure some middle concentration in the Scopometer immediately the reaction is complete and leave it in situ and repeat measurements at short intervals thereafter. Thus the data

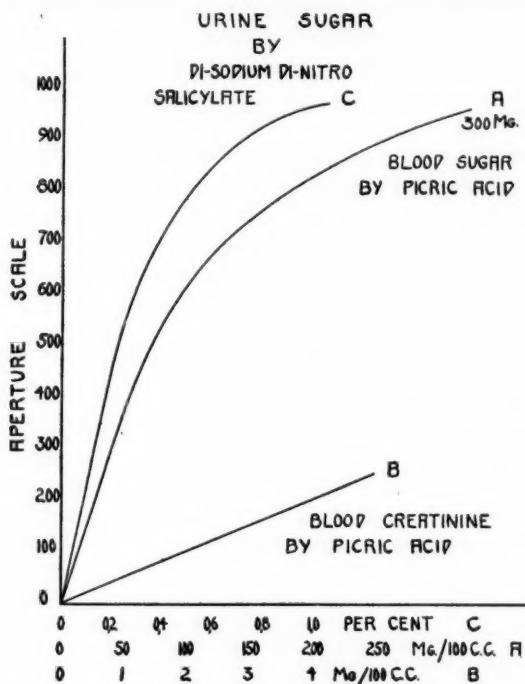


FIG. 5. CALIBRATIONS FOR 3 DIFFERENT METHODS: BLOOD SUGAR BY PICRIC ACID,\* BLOOD CREATININE BY PICRIC ACID,† URINARY SUGAR BY DI-SODIUM DI-NITRO SALICYLATE‡

in Fig. 6 and Fig. 7 indicate that cholesterol must be measured promptly fifteen minutes after completion of the reaction with very little leeway, while

\* Myers, V. C., and Bailey, C. V.: The Lewis and Benedict Method for the Estimation of Blood Sugar, with Some Observations Obtained in Disease. *Jour. Biol. Chem.*, **24**: 147-161. 1916.

† Folin, Otto: On the Determination of Creatinine and Creatine in Blood, Milk and Tissues. *Jour. Biol. Chem.*, **17**: 475-481. 1914.

‡ In press.

hemoglobin may be measured almost any time after fifteen minutes. With this precaution, Calibration Scopometry can attain a very high degree of accuracy because calibrations then cancel out the deviations between simple proportionality and concentration as well as the variables of methods and instrument.

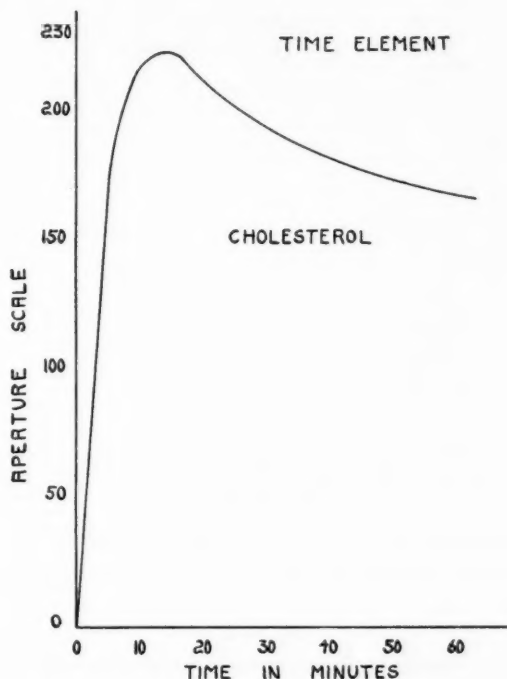


FIG. 6. TIME ELEMENT OF CHOLESTEROL DETERMINATIONS BY ACETIC ANHYDRIDE\*

Note the peak at 15 minutes when measurements of this material should be made. The tints of sample and standard are not always the same but the Scopometer reads them alike.

This may be illustrated by the determinations of six samples of known iron concentration by Rose's<sup>16</sup> micro-method with Scopometer and Duboscq colorimeter shown in table 1.

That these results do not depend upon chance or the skill of any one individual is evident from the results in table 2 which show the application of the

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\* Myers, V. C., and Wardell, E. L.: The Colorimetric Estimation of Cholesterol in Blood. *Jour. Biol. Chem.*, **36**: 147-156. 1918.



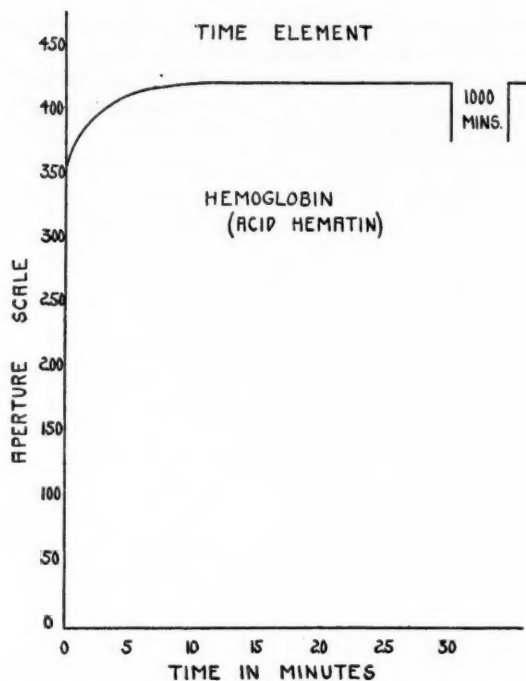


FIG. 7. TIME ELEMENT OF HEMOGLOBIN DETERMINATIONS AS ACID HEMATIN  
Note that this material can be measured any time after 15 minutes

TABLE 1  
MEASUREMENT OF SIX IRON SAMPLES OF KNOWN CONCENTRATION WITH SCOP-  
OMETER AND DUBOSCQ COLORIMETER (TWO STANDARDS)  
(Results given in terms of milligrams Fe per cubic centimeter)

KNOWN CONCENTRA- TIONS	VALUES			DIFFERENCES		
	Scopometer	Duboscq		Scopometer	Duboscq	
		0.06	0.1		0.06	0.1
0.050	0.050	0.049	0.031	0.000	0.001	0.019
0.060	0.057	0.060	0.049	0.003	0.000	0.011
0.080	0.080	0.092	0.076	0.000	0.012	0.004
0.100	0.098	0.128	0.098	0.002	0.028	0.002
0.120	0.123	0.180	0.125	0.003	0.060	0.005
0.200	0.195	0.354	0.222	0.005	0.154	0.022
Average deviations.....				±2%	±16%	±12.5%

TABLE 2  
RESULTS OF IRON ANALYSES OF RAT BODIES BY THE SCOPOMETER METHOD MADE  
AT DIFFERENT TIMES BY TWO ANALYSTS

ANALYST	RAT NUMBER	DATE	SCOPOMETER READING	Fe PER RAT
				<i>mgm.</i>
H	VII F <sub>1</sub>	9/28	395	6.26
			393	6.20
R	VII F <sub>1</sub>	10/24	395	6.26
			395	6.26
H	IV M <sub>2</sub>	9/18	399	6.36
			399	6.36
R	IV M <sub>2</sub>	10/23	400	6.40
			398	6.33
H	III F <sub>1</sub>	10/8	291	1.76
			290	1.74
R	III F <sub>1</sub>	10/9	290	1.74
			291	1.76

All deviations less than 1 per cent.

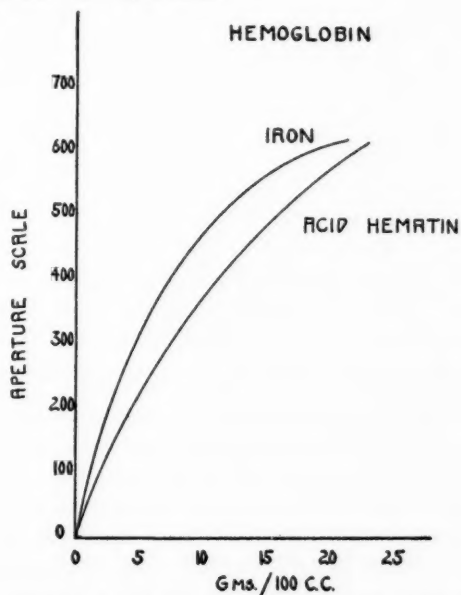


FIG. 8. CALIBRATION CURVE FOR HEMOGLOBIN AS ACID HEMATIN MADE  
DIRECTLY FROM IRON IN BLOOD

same method to a different material by two other analysts working in another laboratory with another Scopometer.<sup>11</sup>

Calibration Scopometry offers some very practical advantages over other kinds of measurements. Perhaps the most important is the ability to measure certain materials which cannot be measured by comparison methods because the materials needed for standards are either unprocureable, too unstable, keep too poorly, or cost too much. This advantage not only saves the trouble and expense of acquiring and maintaining various crude devices with artificial standards for measuring substances like hemoglobin, but also insures far better

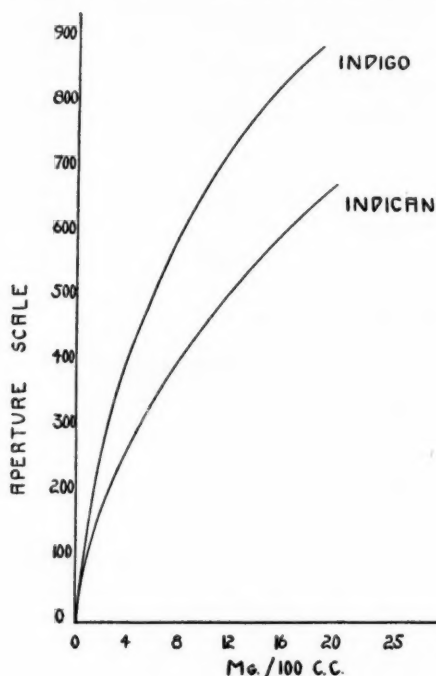


FIG. 9. CALIBRATION FOR INDICAN MADE INDIRECTLY FROM INDIGO

accuracy. To illustrate the application of Calibration Scopometry to such materials, calibrations for measuring hemoglobin<sup>6</sup>, bilirubin<sup>18,17</sup>, indican<sup>15</sup> and protein<sup>3,7,8,5</sup> are shown in figures 3, 9, 10 and 11.

Hemoglobin and its derivatives may be determined with the Scopometer by acid hematin or any other method. Acid hematin has the advantage of being very stable. The Scopometer is calibrated for hemoglobin with native human blood from some healthy individual having a red cell count of at least 5,000,000. In order to cover a range which includes the polycythemias, such a blood sample is concentrated by allowing the cells to settle somewhat and

drawing off the upper layer of plasma. The hemoglobin of this concentrated sample is then calculated from its iron content as determined by Rose's exact micro-method. There is one part of iron to 298 parts of hemoglobin<sup>12</sup>, the non-hemoglobin iron being negligible, i.e., less than 5 parts in 10,000.

The upper curve in figure 8 shows the calibration for iron in blood in terms of hemoglobin. The iron in the blood sample used for the acid hematin calibration shown in the lower curve of figure 8 gives a hemoglobin equivalent of 23 grams per 100 cc. A mixture of 5 cc. of the blood sample and 5  $\frac{1}{4}$  cc. normal saline gives 20 grams of hemoglobin per 100 cc., a convenient unit for the sub-sample dilutions. Thus a 1-1 dilution gives 10 grams, a 1-3 dilution 5 grams,

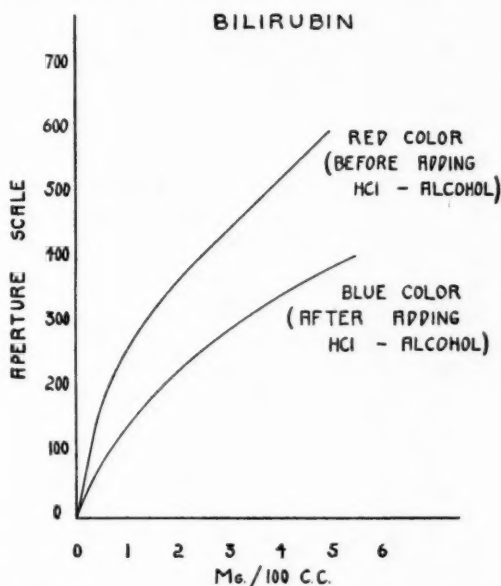


FIG. 10. CALIBRATION CURVE FOR BILIRUBIN WITHOUT REFERENCE TO THE VARIOUS ARTIFICIAL STANDARDS USUALLY EMPLOYED

and 3 parts blood and 1 saline 15 grams hemoglobin. Other portions of the sample are similarly diluted to obtain other concentrations. To 0.1 cc. of each of these subsamples 20 cc. of 0.1 N HCl are added and the mixture allowed to stand 20 minutes and then read in the Scopometer. The scale readings are plotted on the ordinate against the concentrations on the abscissa as in figure 8. Subsequent determinations are made by treating unknown samples with HCl as above indicated and referring the aperture scale readings to the calibration curve.

A calibration for bilirubin is best made directly from a mixture of bilirubin or human bile and plasma (or serum). Enough bilirubin or bile is added to a

clear sample of plasma to have a concentration which corresponds with the highest bilirubin content of interest. Portions of this are diluted down to secure the desired concentrations of bilirubin and to each of these in turn is applied Van den Bergh's method or some modification which may be preferred. In figure 9 are shown calibrations of the red material which results from simple diazo coupling according to Van den Bergh<sup>18</sup> and the blue material obtained by treating the red material with HCl-alcohol after Thannhauser and Anderson.<sup>17</sup> This simple and direct calibration made under exactly the same con-

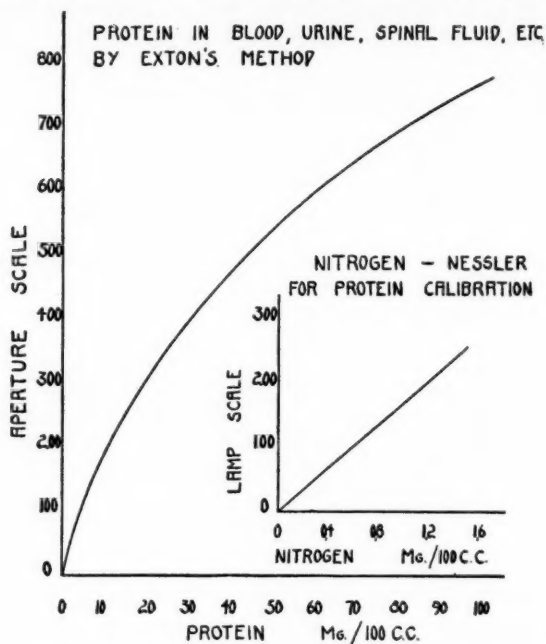


FIG. 11. CALIBRATION FOR PROTEIN IN BLOOD, URINE, SPINAL FLUID, ETC., BY EXTON'S METHOD BASED ON NITROGEN DETERMINATION TOGETHER WITH CALIBRATION FOR NITROGEN BY NESSLER

ditions as bilirubin occurs in blood seems to eliminate the color and other difficulties which have been encountered in quantitative bilirubin estimations.

Indican illustrates how the Scopometer can be calibrated indirectly for materials which are not only exceedingly difficult to obtain but very unstable. Select a urine sample having a relatively high concentration of indican. Convert the indican into indigo and measure the indigo by its aperture scale reading on a Scopometer which has already been calibrated for indigo. The indigo value thus obtained multiplied by 0.82 gives the concentration of indican. Convenient dilutions are then made as in the case of hemoglobin and the

TABLE 3

STUDY OF FLOCCULATION EFFECTS OF ACID AND ALKALI CONCENTRATION ON  
PURIFIED GLYCOPROTEIN

Note that turbidity increases with acid, clears with alkali and reappears on subsequent addition of acid; also the effects of sodium acetate on the nature of the flocculation.

	DROPS 0.1 N ACID-ALKALI	SCOPOMETER READING	PROTEIN <i>mgm. per 100 cc.</i>
Acid	1 (faintly turbid)	178	8.8
	2	288	16.5
	3	373	23.9
	4	414	28.0
	5	496	38.6
	6	520	42.6
	7	560	49.5
	8	598	57.6
	9	641	67.2
	10	684	81.6
	11	721	94.3
	12	746	105.6
	13	773	118.8
	14	793	133.8
	15 (flocks)	795	135.0
Alkali	16	796	135.8
	1	796	135.8
	2	786	128.5
	3	772	118.8
	4	752	108.5
	5	745	105.0
	6	730	98.0
	7	715	92.3
	8	698	86.2
	9	670	76.2
	10	646	68.3
	11	576	52.4
	12	488	37.4
	13	282	16.5
	14	140	6.5
	15	131	6.0
	16 (clear)	128	5.8



TABLE 3—*Concluded*

	DROPS 0.1 N ACID-ALKALI	SCOPOMETER READING	PROTEIN <i>mgm. per 100 cc.</i>
Acid	1	130	5.9
	2	130	5.9
	3	132	6.0
	4	155	7.3
	5	186	9.1
	6	242	13.0
	7	287	16.3
	8	348	21.3
	9	410	27.6
	10	451	32.5
	11	505	40.0
	12	530	44.2
	13	551	48.1
	14 (opalescent)	568	50.6
	15	583	54.2
	16	590	56.0

indican in each dilution is determined by Rose's<sup>15</sup> method. The scale readings are then plotted against concentrations as in the case of hemoglobin. Subsequent determinations of indican are made by applying Rose's method to the unknown sample, reading the result in the Scopometer and picking the value of the sample from the indican curve.

The Scopometer is calibrated for protein directly from the nitrogen in a sample of plasma determined by Rose's<sup>14</sup> convenient micromethod and Nesslerization. The nitrogen is then determined with either the Calibration or Comparison Scopometer. In the case of the calibration shown in figure 11, the plasma contained 1010 mgm. nitrogen per 100 cc. When 30 mgm. non-protein-nitrogen were deducted the protein nitrogen was 980 mgm. per 100 cc. Multiplying this by the factor 6.35 gave the total protein value of the plasma sample as 6223 mgm. per 100 cc. Therefore, 1 cc. of the plasma diluted to 62.2 cc. with normal saline had a protein content of 100 mgm. per 100 cc. which is a convenient unit for diluting the subsamples in steps of 10 mgm. protein. The turbidities resulting from mixtures of equal parts of the subsamples and of Exton's reagent are read in the Scopometer and the scale readings plotted against concentrations as in the foregoing illustrations. Subsequent determinations of the protein in blood, urine, spinal fluid, etc., are then made by precipitating the protein of the unknown samples by Exton's method and picking the values from the calibration curve. The turbidity of this precipitate is as reproducible as the colors of the best colorimetric methods.

There are also certain materials which cannot be measured by comparison

methods because they do not follow any definite law, although constant in their irregularities. The ability to measure such materials is another advantage of Calibration Scopometry.

Still other advantages of Calibration Scopometry flow from its speed and ease of operation which make practicable for routine work certain methods which are otherwise impracticable because too time-consuming and laborious. Typical of such instances is the very valuable disodium dinitro salicylate sugar reduction rate method which not only quantitates but also indicates the nature of reducing substances in urine. (See calibration in figure 5.) Besides these

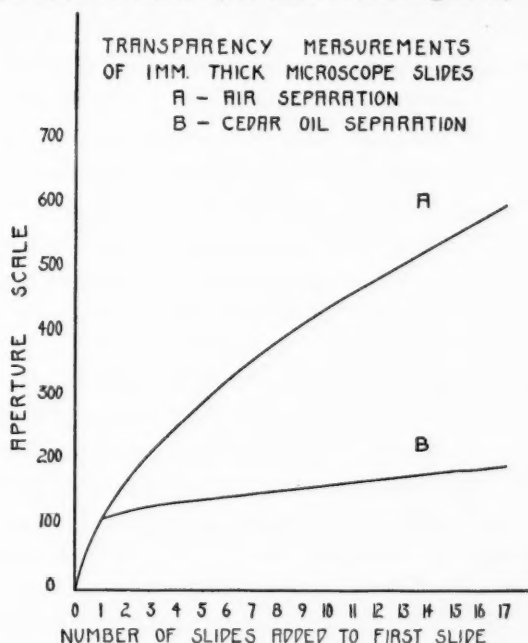


FIG. 12. TRANSPARENCIES OF 17 NEW CLEAR GLASS BAUSCH AND LOMB MICROSCOPE SLIDES AS DETERMINED BY THE SCOPOMETER

special advantages, Calibration Scopometry makes extremely delicate titrations practicable, as illustrated by the study of the solubilities of a glyco-protein preparation by turbidity shown in table 3.

For certain other special purposes, like standardizing vaccines and other materials, sometimes with permanent standards, the precision of the Calibration Scopometer may be illustrated by its use as a densitometer in a study of chemical fog in X-ray films by a physicist. Thus Dr. Wells reports:

"The average deviation of 153 measurements made over a period of three months on nine film standards ranging from 80 per cent to 3 per cent in trans-

mission was 0.02 Scopometer scale divisions. This deviation is constant over the whole range. The Scopometer thus measures chemical fog to 0.002 in density, which is about twice as accurate as visual densitometers."

The extreme sensitivity of the instrument in the low range where sensitivity is most useful may be illustrated by transparency measurements of clear glass shown in figure 12.

#### INDICATIONS FOR CALIBRATION AND COMPARISON SCOPOMETRY

Besides the applications outlined above, Calibration Scopometry measures materials which it has hitherto been customary to measure with nephelometers, or turbidimeters and similar instruments much better than has ever been possible with visual instruments. In a previous paper<sup>4</sup> I have discussed some of the reasons that led a physicist to declare "apparently turbidity methods have not proved satisfactory"<sup>19</sup> and also stated conditions which might be counted on to make turbidity measurements more satisfactory. It may therefore be noted that Calibration Scopometry fulfills these conditions and is unquestionably the measurement par excellence for turbid media.

In addition to the foregoing advantages, Calibration Scopometry is the quickest, simplest and most economical of all measurements because it gives results directly in terms of concentration, and saves the time, trouble and expense of preparing comparison standards. *Calibration Scopometry, therefore, is indicated whenever an appropriate calibration is at hand and the material to be measured is suitable for calibration measurements.*

Some materials are not suitable for calibration measurements because they are products of reactions in which some chemical, physical or environmental factor affects the results so that they do not always transmit (or absorb) an identical amount of light. In these instances the same materials may not measure the same from one day to another, or from one batch of reagent to another, when referred to pre-determined calibrations.

This tendency to shifts in optical value seems more apt to occur when the chemistry of a method involves extreme niceties of adjustment or complex reagents which change with time or differences in the particle sizes of colored colloids. Typical of such conditions are the alkaline and copper molybdc reagents of Folin's blood sugar method and Nesslerization.

Although unsuitable for calibration measurements, the materials in question give satisfactory results with comparison measurements. This happens because the shifts in optical value to which they are subject are constant for each determination and affect standard and unknown samples alike. Such shifts therefore cancel out when the unknown samples are compared with standards made of the same materials treated in the same way at the same time as is the practise in Comparison Scopometry and Duboscq colorimetry. *Not needing calibrations, the indications and applications of Comparison Scopometry and Duboscq colorimetry are exactly the same.*

#### COMPARISON SCOPOMETRY

Comparison Scopometry is a new measurement of light transmission (or absorption). In principle it measures the distance between light source and sample in terms of 0.5 mm. scale divisions and determines the concentration of an unknown sample by relating its scale reading to those of similar samples of known concentration.

Two standards, one low and one high in the pathological range, are run simultaneously with the unknown sample or samples as in Duboscq colorimetry, and their measurements mark the slope of a straight line on which the unknown samples will fall.

This happens because the proportionality between scale readings and concentrations is nearly linear and the deviations so slight and regular that a simple scale adjustment effectively continues linearity over the whole range. This adjustment is shown in figure 13 and it is understood in all subsequent references to comparison measurements that scale readings mean adjusted scale readings.

Preliminary to operation, light the galvanometer lamp and see that the galvanometer registers "0" before snapping on the power switch which lights the Scopometer lamp and pulling the push-pull switch out to throw in the photo-electric circuit.

Operation begins by null-setting the system with the low standard in both cups. Cups are best placed symmetrically nearest the photo tubes.

#### *To Null Set*

*With filled cups in place and lamp on "0" (extreme left) balance the system by turning the aperture dial until the galvanometer rests on "0".*

*To Measure*

*With low standard in left cup substitute the high standard and unknown samples in right cup and balance the system by turning the lamp dial until the galvanometer rests on "0". Read the adjusted scale.*

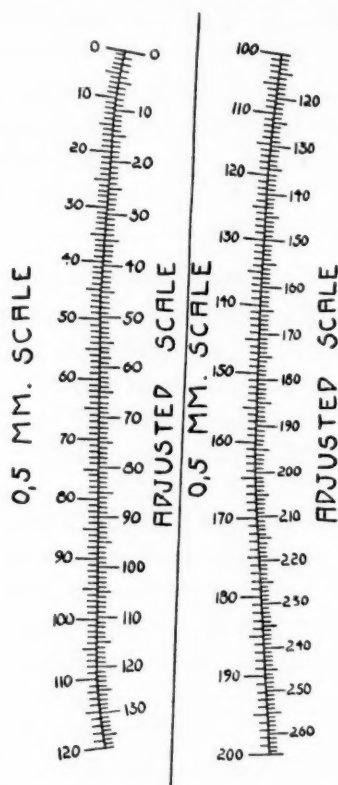


FIG. 13. ADJUSTMENTS OF CALIBRATION SCOPOMETRY SCALE TO 0.5 MM. SCALE ON LAMP MOVEMENT DIAL

A simple calculation then gives the concentration of the unknown samples. If  $C$  = concentration;  $R$  = adjusted scale reading; and subscripts 1 and 2 and  $x$ , low and high standards and the unknown sample, respectively, then

$$Cx = \frac{Rx(C_2 - C_1)}{R_2} + C_1$$

The simple arithmetic of this straight line equation may be illustrated by actual data. Thus,  $XY$  and  $Z$  are the three blood samples of a sugar tolerance test.  $W$  and  $L$  samples from other individuals and  $C_1$  and  $C_2$  low and high

standards of known concentration which were run at the same time by the Folin-Wu blood sugar method. The scale readings are as follows:

$C_1$  reads 0, i.e., low, (100 mgm.), standard used for null-setting the instrument

$C_2$  reads 142, i.e., high, (300 mgm.), standard

$X$  reads 68

$Y$  reads 104

$Z$  reads 175

$W$  reads 11

$L$  cannot be read because its color density is less than  $C_1$ , the low (100 mgm.) standard.

Substituting these scale readings for the symbols of the equation gives the following results:

$$\begin{array}{ll} \text{Sample } X: R_x = 68 & C_1 = 100 \\ R_2 = 142 & C_2 = 300, \text{ i.e.,} \end{array}$$

$$C_x = \frac{68(300 - 100)}{142} + 100 = 196 \text{ mgm. per 100 cc.}$$

$$\begin{array}{ll} \text{Sample } Y: R_y = 104 & C_1 = 100 \\ R_2 = 142 & C_2 = 300, \text{ i.e.,} \end{array}$$

$$C_y = \frac{104(300 - 100)}{142} + 100 = 247 \text{ mgm. per 100 cc.}$$

$$\begin{array}{ll} \text{Sample } Z: R_z = 175 & C_1 = 100 \\ R_2 = 142 & C_2 = 300, \text{ i.e.,} \end{array}$$

$$C_z = \frac{175(300 - 100)}{142} + 100 = 347 \text{ mgm. per 100 cc.}$$

$$\begin{array}{ll} \text{Sample } W: R_w = 11 & C_1 = 100 \\ R_1 = 142 & C_2 = 300, \text{ i.e.,} \end{array}$$

$$C_w = \frac{11(300 - 100)}{142} + 100 = 116 \text{ mgm. per 100 cc.}$$

Sample  $L$ . The concentration of  $L$  is evidently less than  $A$  (the 100 mgm. low standard). In all cases where the concentration of an unknown sample (like  $L$ ) runs less than the low standard, treat the low standard (whatever it may be) as the high standard and use clear water for the low standard, i.e., null-set the instrument with clear water. In the case of



$$\begin{array}{ll} \text{Sample L: } R_2 = 62 & C_1 = 0 \\ & R_2 = 92 \quad C_2 = 100 \end{array}$$

$$C_L = \frac{62(100 - 0)}{92} + 0 = 68 \text{ mgm. per 100 cc.}$$

The concentrations of unknown samples will rarely or never run higher than the scale of the instrument set with well chosen standards. If they do, one has a choice between two alternatives. The simpler procedure is to bring the samples within range of the scale by diluting them 1-1 with water or re-

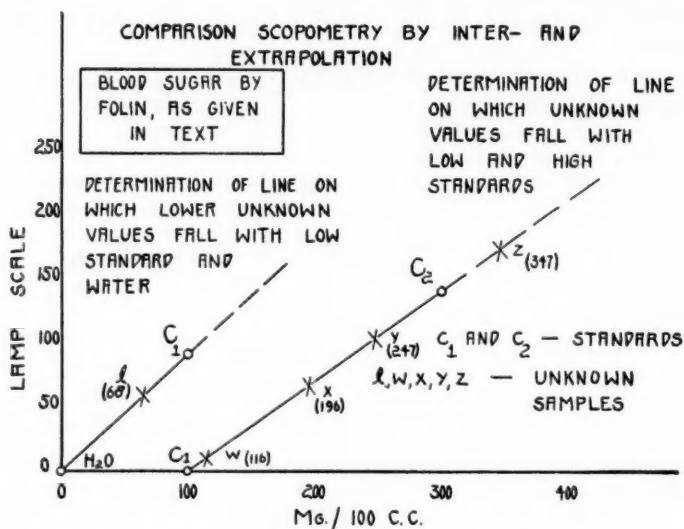


FIG. 14. COMPARISON SCOPOMETRY BY INTER- OR EXTRAPOLATION

A quick method useful when running a series of similar determinations. Note that the results are the same as calculated by arithmetic.

agent. The more accurate procedure is exactly the same as the original with higher standards. In this connection it may be noted that the chemistry of many methods does not hold true in the extreme high and low ranges. Thus, the authors specify that the limits of the Folin-Wu blood sugar method are 70-400 mgm.

The same results are also conveniently obtainable by graphic interpolation or extrapolation instead of by arithmetic, and the same data treated in this way are shown in figure 14. It is a simple matter on cross-section paper to plot scale readings on the ordinate against concentrations on the abscissa. Whatever it may be, the low standard is always pointed off as 0 on the chart and the high standard is pointed off at the intersection of its scale reading with

its known concentration. All of the unknown samples fall on a straight line drawn through these points and extended across the chart. Their concentrations are, therefore, found on the abscissa in line with the points where their scale readings meet the straight line. The calculations by either method

TABLE 4  
BLUE. FOLIN GLUCOSE

ACTUAL CONCENTRATION	CALCULATED CONCENTRATION		DEVIATION	
	Duboscq	Scopometer	Duboscq	Scopometer
<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>per cent</i>	<i>per cent</i>
60	58.5	59.6	-2.5	-0.7
80	77.0	81.0	-3.7	+1.2
100	Standard	Standard		
150	146	150.7	-2.7	+0.5
200	Standard	Standard		
250	245	250	-2.0	0
300	284	295	-5.0	-1.6
350	320	335	-8.5	-4.5
400	357	388	-11.0	-3.0
Average.....			±5.1	±1.7

TABLE 5  
GREEN. CHOLESTEROL

ACTUAL CONCENTRATION	CALCULATED CONCENTRATION		DEVIATION	
	Duboscq	Scopometer	Duboscq	Scopometer
<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>per cent</i>	<i>per cent</i>
100	96	100	-4.0	±0.0
200	Standard	Standard		
300	281	304	-6.3	+1.3
400	385	401	-4.0	+0.2
500	505	495	+1.0	-1.0
800	Standard	Standard		
1000	990	990	-1.0	-1.0
Average.....			±3.3	±0.7

and the manipulations of Comparison Scopometry take less time than does Duboscq colorimetry.

The relative accuracy of Comparison Scopometry and Duboscq colorimetry may be illustrated by measurements of the same materials of known value by

both instruments. The measurements were made with a Bausch and Lomb biological model colorimeter in perfect condition by an expert with long train-

TABLE 6  
YELLOW. NITROGEN BY NESSLER

ACTUAL CONCENTRATION	CALCULATED CONCENTRATION		DEVIATION	
	Duboscq	Scopometer	Duboscq	Scopometer
<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>per cent</i>	<i>per cent</i>
0.2	0.212	0.205	+6.0	+2.5
0.3	0.305	0.296	+1.6	-1.3
0.4	Standard	Standard		
0.5	0.490	0.495	-2.0	-1.0
0.6	0.586	0.606	-0.7	+1.0
0.8	Standard	Standard		
1.0	1.040	1.003	+4.0	+0.3
1.5	1.490	1.475	-0.8	-1.3
2.5	2.550	2.517	+2.0	+0.8
Average.....			±2.4	±1.2

TABLE 7  
RED. FUCHSIN SOLUTION

ACTUAL CONCENTRATION	CALCULATED CONCENTRATION		DEVIATION	
	Duboscq	Scopometer	Duboscq	Scopometer
<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>per cent</i>	<i>per cent</i>
0.05	0.04	0.049	-25.0	-1.0
0.10	0.101	0.099	+1.0	-1.0
0.25	Standard	Standard		
0.55	0.62	0.560	+11.3	+2.0
0.80	0.89	0.820	+10.0	+2.5
1.00	Standard	Standard		
1.50	1.66	1.500	+10.0	+0
Average.....			±11.5	±1.3

*Summary of Duboscq colorimeter—comparison scopometer data*

	<i>Duboscq</i>	<i>Scopometer</i>
Average deviations from 24 actual concentrations.....	5.5%	1.2%
Extreme plus deviations.....	11.5%	2.5%
Extreme minus deviations.....	25.0%	4.5%

ing in the use of the colorimeter and very little experience in the use of the Comparison Scopometer. The data were obtained on blue, green, yellow and red materials of everyday interest, i.e., blood sugar by Folin, Non-Protein

Nitrogen by Nessler, cholesterol by acetic anhydride, and a red solution of fuchsin which was chosen because its transmission is in the region where the photo tubes are least sensitive.

The superior precision of the Comparison Scopometer is in part, at least, due to a condition which is well known but often forgotten in practise. In Duboscq colorimetry the range over which Beer's law holds good is limited and varies with different materials. For this reason, apparent in the foregoing data, errors are likely to occur which mount rapidly in magnitude when standards and unknown samples do not approximate one another closely enough. Comparison Scopometry has no such limitations because it is not based on Beer's law.

Another advantage of Comparison Scopometry over Duboscq Colorimetry is the matter of lost specimens. In Duboscq Colorimetry the chemistry and measurement of all samples must be simultaneous. The loss of any specimen in a series therefore necessitates repetition of the bench work on all of them. In Comparison Scopometry, on the other hand, the loss of a specimen necessitates only a repetition of the bench work on the lost specimen at one's convenience.

#### DISCUSSION

It is customary in descriptions of photo-electric measuring devices to emphasize the improvement in precision which results from the use of an objective electrical criterion instead of a subjective visual one. Thus, it is well-known that slight and temporary changes in mental or physical condition affect vision and that not only the eye but the brain back of it are subject to physical and psychical variations which may impair the accuracy of visual measurements. Besides avoiding such sources of error, it appears from comparisons of Scopometric with visual data that the Scopometer's accuracy is greater than the mere improvement attributable to the difference between subjective and objective criteria. In part, at least, this is due to the fact that the photo tubes of the Scopometer are more sensitive than human eyes to differences in light intensity, but certain features of design and construction which distinguish the Scopometer from other devices also account for this superiority. One of them is the dominating functional stability of its photo-electric tubes. Another is its symmetry, optical and electrical, which cancel out fluctuations in current supply and differences in the characteristics of galvanometers and photo-electric tubes. Above all, however,

the Scopometer gains sensitivity and precision by measuring light not in terms of electrical units but in terms of light, i.e., by the area of its variable aperture (Calibration Scopometry) or the position of its light source (Comparison Scopometry).

From these features also flow the remarkably simple, speedy and comfortable operation of the instrument which novices master in a few minutes and then measure unprecedented numbers of samples in a given time with no fatigue at all. In conclusion, I desire to record the debt and gratitude I owe others for their help in the development of the Scopometer, especially Dr. H. C. Rentschler for his coöperation and assistance with photo-electrics; Mr. Carl Keuffel for his assistance with optics; Mr. P. H. Rader for perfecting the mechanism of the variable aperture; and Mr. H. D. Sheldon for his fine coöperation in the details of construction.\*

Thanks are also due the members of my staff for their coöperation and interest, and I am especially obligated to Dr. Fred Schattner and Miss Mary C. McCarthy for excellent technical assistance; to Dr. Edward J. Roehl for assistance with mathematics and charts; and to Mr. John Huizer for supervising routine work with the Scopometer. Above all I am grateful to Dr. Anton R. Rose for his enthusiasm, critical testing, and valuable suggestions which have so greatly contributed to the success of the Scopometer.

#### SUMMARY

A new photo-electric instrument for measuring the light transmissions (or absorptions) of colored and turbid media has been described. It is called the Universal Electro-Scopometer because it is unlimited in its applications to every laboratory demand and contingency for such optical measurements.

This unique flexibility results from combining two different methods of measurement in one instrument: a substitution method with pre-determined calibrations and a direct method

\*The matter of making the Electro-Scopometer available to others I have entrusted to Mr. H. D. Sheldon, 323 Belleville Avenue, Bloomfield, N. J., who is in a position to supply instruments made according to my specifications.

with comparison standards. The principles, construction and operations of the instrument are described, the indications and applications of Calibration and Comparison Scopometry are explained, and the superior precision, range and sensitivity of the Scopometer are illustrated by data.

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## THE PATHOLOGY OF THE NEWER COMMERCIAL SOLVENTS\*

A. V. ST. GEORGE

*From the Pathological Laboratories of Bellevue Hospital, New York*

The introduction into industry of a whole series of non-explosive volatile solvents to replace the highly inflammable and explosive type, while dissipating to a great extent the hazards and dangers of explosions and fires with their subsequent effects, has nevertheless introduced an added industrial hazard and new forms of occupational poisoning.

These solvents are used in a great many diversified industries. One need but mention the dyeing and cleaning industry, the rayon and other nitrocellulose, acetylcellulose and artificial leather factories; the manufacture of varnish and paint, and as a solvent for gums, resins, waxes, rubber and pharmaceuticals. As fumigants and insecticides, many of them are found of value, and even in the manufacture of artificial pearls at least one is used. Often these substances are added to a compound to soften it or produce a plastic mass; sometimes they add lustre or a glaze to a surface, and at times will insulate the material.

Experimental evidence concerning the toxicity of these solvents has been reported from time to time. In practice, however, conditions different from those that obtain in the experimental laboratory are encountered. Thus in industry generally, technical instead of chemically pure chemicals are used; again, many times two or more of the solvents are employed in combinations or at the same time, thus further complicating the clinical, post-mortem and toxicological picture and the resulting medico-legal aspects of a case. A clear cut symptomatology therefore is

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generally not possible. It must, however, be emphasized that many of these compounds are so closely related that the clinical picture presented is similar and only by chemical analysis can a differentiation be made.

The toxicological importance of these solvents is not only that they produce acute and fatal poisonings, but because they often cause (slow) chronic intoxications which are difficult of recognition. In acute cases with a fatal termination it is possible to isolate from the cadaver and identify the poison by means of the micro-methods devised by Gettler, Niderl and others. On the other hand, chronic poisonings are much more difficult to identify. Small amounts continually introduced in the body are believed to be fairly rapidly eliminated through the kidneys and lungs in just such minute amounts, and hence it is practically impossible to detect them. The possibility of their being broken up into other compounds and retained in the body for a time has not been accurately determined. We must therefore rely on the clinical picture and pathological findings for our determinations.

In general these poisons have a varying degree of irritability on the mucous membranes of the conjunctivae, respiratory and gastro-intestinal tract. They also have an effect on the central nervous system, probably chiefly because of their lipid solvent activity, and in certain cases on the liver, kidneys, etc. Practically all of them effect the hemopoietic system.

*Methyl acetate:* In experimental animals methyl acetate produces a moderate irritation of the mucous membranes and has a slight narcotic action. Continued inhalation of small amounts produces somnolence and prolonged headaches together with changes in the blood. The hemoglobin, red cells and leucocytes are increased together with diminution or disappearance of the eosinophiles and a relative increase in the polynuclear neutrophiles. The platelets may be increased. When large amounts are inhaled the substance leads to narcosis, abolition of the reflexes, and death with the symptoms of a narcotic poison. No known human death from methyl acetate has been reported. In commercial practice methyl acetate is frequently mixed with alcohols, ether, benzine, etc., and many of the cases of poisoning are undoubtedly due to an admixture with one of these.

Post-mortem examinations on animals show generalized congestion, diminished coagulation of the blood, and submucosal hemorrhages.

*Ethyl acetate:* Ethyl acetate is a moderate irritant of the mucous membranes and is a somewhat stronger narcotic than methyl acetate. Continued inhalation of the product in sub-narcotic concentrations causes loss of appetite, loss in weight, and blood changes in which there is an increase in the erythrocytes and the leucocytes without an increase in the percentage of hemoglobin. The symptomatology is essentially the same as that produced by methyl acetate.

Althoff<sup>1</sup> reported a case of death in a man which followed the painting of the inside of a tank wagon with a varnish containing 80 per cent ethyl acetate. Necropsy showed marked congestion of the viscera and the eyes; almost entirely liquid blood in the vessels; petechial-like hemorrhages throughout the serous cavities and mucous membranes and a pungent odor on opening the body.

*Amyl acetate:* Amyl acetate, commonly referred to as banana oil, was formerly most frequently used in light varnishes. Most people object to its sweet, sickening odor and following inhalation of its vapors have complained of drowsiness, irritation of the throat, spasms of coughing, burning eyes, vertigo, tachycardia, tinnitus aureum, nausea and gastric irritation. In animals, chronic inflammatory changes in the respiratory tract, parenchymatous degeneration of the kidneys and fatty livers have been found. Changes produced in the blood are secondary anemia with a moderate leucocytosis. It has a stronger narcotic action than the lower esters of acetic acid but in experimental animals the narcosis is better borne. Pure amyl acetate is more harmful than butyl acetate. Creelius<sup>2</sup> reported one death in a man in whose body edema of the glottis and diffuse irritation of the respiratory and gastro-intestinal tract were the chief findings.

*Propyl acetate:* Propyl acetate is a moderate irritant of the mucous membranes, but its narcotic action is equal to that of ethyl ether, thus it is far more narcotic than either methyl or ethyl acetate. On the other hand in lethal concentrations it is less toxic than either one. Post-mortem examinations show hyperemia and congestion especially in the respiratory tract, congestion of the viscera, and fat deposits in the liver. The blood cells and hemoglobin show little, if any, change. A slight leucocytosis may be found.

*Butyl acetate:* Butyl acetate has a much stronger irritating action on the mucous membranes than any of the lower homologues. The irritation is especially marked in the conjunctivae, frequently leading to conjunctivitis. Its narcotizing action is likewise more marked. Prolonged inhalation of dilute concentrations result in loss of appetite and weight, and an increase at first in the hemoglobin, red blood cells and the leucocytes, without changes in the structure of the cells. Later the red cells and hemoglobin fall, but the leucocytosis becomes more marked. The patient likewise complains of great fatigue, giddiness and headaches and frequently abdominal pains. In its lethal action it is less poisonous than methyl or ethyl acetate and somewhat less damaging than amyl acetate. Post-mortem examinations in experimental animals show congestion of the viscera, hemorrhages throughout the mucous membranes and

at times in the skin. Post-mortem clotting is almost absent. In man no death has been recorded.

*Benzyl acetate:* No cases of benzyl acetate poisoning or industrial injury have been reported in the literature up to 1934. Because of its slow volatility it requires a good deal to induce appreciable irritation of the mucous membranes. For the same reason its narcotic action is slight. Its action on the central nervous system and the blood must be considered, producing tremors, headaches and severe anemia. In experimental animals it is found to be toxic to white mice, but is well borne by cats. A suspected case of industrial poisoning by benzyl acetate was studied by the writer a number of years ago. The patient showed marked tremors, some ataxia, and profound changes in the blood cells. He had, however, a number of years previously worked with benzene and because of the similar blood picture in the patient, it was felt that the latter was probably the provocative agent.

*Diethylphthalate:* Diethylphthalate when volatilized produces marked irritation of the mucous membranes. When injected into animals it produces paralysis of the central nervous system. It is not easily volatilized and thus will produce irritative lesions of the skin, especially the hands and face. When sprayed it is inhaled and produces acute symptoms, such as respiratory difficulties, hemorrhagic nephritis, fatty metamorphosis of the liver, diminution of the hemoglobin with an increase in the red and white cells, and a relative polynucleosis with loss of the eosinophiles.

*Methyl glycol acetate:* Methyl glycol acetate possesses only a slight narcotic and irritative action on the mucous membranes. It is by no means harmless, being ten times more toxic than methyl alcohol, producing similar symptoms and lesions. It destroys both the hemoglobin and red cells and, to a certain extent, the white blood cells.

*Acetone:* Acetone is much less irritating than the acetic acid esters. It is a narcotic and even more so than chloroform. The impurities cause some of the dangerous symptoms which are encountered. Prolonged inhalation causes destruction of the hemoglobin and red blood cells, without appreciable change in the leucocytes.

*Ethylene glycol:* Ethylene glycol is not narcotic, is slowly vaporized and produces only slight irritation of the mucous membranes. No death resulting from this substance has been recorded.

*Carbon bisulphide:* Carbon bisulphide was formerly an important ingredient in the vulcanizing industry but because of its highly toxic and inflammable properties was gradually eliminated. Recently, however, it has found increasing use in the rayon industry and again cases of poisoning have appeared. It is primarily a poison to the central nervous system, causing ataxia, tremors, mental confusion and atrophy of the optic nerve. The blood picture shows varying degrees of anemia with only occasional changes in the red blood cells and rarely a leucocytosis. Post-mortem examinations show but little to the

naked eye, except for hemorrhages and congestion, and fat deposition in the liver. Histologically, atrophic lesions and focal necroses in the brain are found.

*Carbon tetrachloride:* Carbon tetrachloride produces both acute and chronic poisoning. Chronic cases occur as the result of accidental exposure to or escape of the fumes. The symptoms are usually initiated by abdominal pains, vomiting, headache, vertigo, and if exposure is prolonged, coma, edema, urinary suppression, jaundice and hepatitis. Changes in the blood show secondary anemia with leucocytosis and relative polynucleosis. On post-mortem examination, congestion of the viscera, acute parenchymatous degeneration of the kidneys, hepatitis with fat necrosis and jaundice have been found. Its action on the liver is profound, as was shown in the experimental work of Bollman and Mann.<sup>3</sup> Lyon<sup>4</sup> reported a case of cirrhosis of the liver which is believed to have been caused by the long continued use of this product in a hair tonic. Additional interesting cases are mentioned by Alice Hamilton.<sup>5</sup>

*Ethylene chlorbromide, dibromide and dichloride:* These products are all toxic in varying degrees of concentration, especially when inhaled, producing irritation of the mucous membranes, headache, vertigo, giddiness, nausea, vomiting, drowsiness, coma. They invariably in sub-narcotic doses produce anemias, usually quite marked. On post-mortem examination especially when inhaled, congestion and edema of the lungs and degenerative changes in the kidney are the prominent lesions. A case of ethylene dibromide poisoning was described by Kochmann<sup>6</sup> in which there was marked inflammation and swelling of the eye-lids, pharyngitis, bronchial catarrh, anorexia, headache and weakness. Death in experimental animals was caused by circulatory failure and paralysis of the central nervous system. Fortunately these latter fluids have a distinct odor so that the worker can be warned of impending danger.

*Trichlorethane:* (incorrectly called ethylene trichloride). Trichlorethane is an acute narcotic poison. It produces lesions in the skin resembling burns, and causes acute congestion of the lungs, kidneys, liver and spleen. In experimental animals it produces rapid anaesthesia with diminution of the hemoglobin, secondary anemia and relative polynucleosis. After the death of the animal (guinea-pigs) in addition to the above findings, some fatty metamorphosis of the liver has been noted.

*Trichlorethylene:* Trichlorethylene, because of its solvent action on fats and oils, is used as a thinner for coatings and varnishes and a solvent for rubber, replacing carbon tetrachloride in many instances. It is frequently recommended as a non-poisonous solvent, but evidence, chiefly adduced by the Germans, shows it to be far from innocuous. Twenty-six deaths were recorded by Stuber<sup>7</sup> in which the lesions were those of phosgene. The liquid is a more powerful narcotic than carbon tetrachloride, but unlike the latter, it exerts its effect on the central nervous system, giving paralysis of the sensory portion of the 5th cranial nerve and optic nerve atrophy. Capillary injuries, especially in the cerebral vessels, are thought to occur but thus far have not been demon-



strated. Usually in the chronic cases marked secondary anemia and moderate increase in the leucocytes may occur but no changes in the morphology of the cells has been noted.

*Tetrachlorethylene*: Tetrachlorethylene is a relatively new solvent. Its low volatility and the fact that it is practically not absorbed from the intestinal tract is of some therapeutic (anthelmintic) value. Upon inhalation, symptoms of nausea, giddiness, and vomiting may ensue together with subsequent slight irritation of the conjunctivae, headache, drowsiness. Changes in the blood cells have not been recorded by any observers up to the present time.

*Acetylene tetrachloride also known as tetrachlorethane*, is the most poisonous of the chlorinated hydrocarbons, but also the most useful in industry, particularly in the production of cellulose acetate. It produces acute and chronic poisoning. In the former, the symptoms develop rapidly and are those of acute yellow atrophy of the liver, fatty changes in other viscera, especially the heart, hemolysis, jaundice and death. In the chronic cases, emaciation, mononucleosis, hemolysis, secondary anemia with stippling of the red cells, hemoglobinuria, and even jaundice may develop.

*Acetylene tetrabromide*: Acetylene tetrabromide has not found much application in industries as yet. It is used in liquid gauges and in the separation of ores. It is toxic to experimental animals on exposure in strong concentrations.

*Monochlorobenzene, orthodichlorobenzene, trichlorobenzene*: These chlorinated compounds of the benzene ring are not more toxic than benzene, thus differing widely from the introduction of chlorine into the aliphatic series or a nitro group into the aromatic ring. The addition of a nitro group to a chlorobenzene compound (nitrochlorobenzene) however, greatly increases its toxicity. It is important to note that while the derivatives of the benzene ring cause poisoning by inhalation of the fumes, by far the greater number of cases are produced by absorption through the skin. The need for scrupulous cleanliness with ample washing and bathing facilities in factories using these substances is obvious.

*Orthodichlorobenzene* is used in a spray as a fumigant either alone or in combinations, and in admixtures with paints producing a "fumigating" paint used in painting stables, chicken houses, granaries, etc. If care is taken in its use it does little harm to human beings. On the other hand exposure in a confined space produces irritation of the respiratory tract, lacrimation, vertigo, etc., in both man and animals. Continued exposure produces considerable anemia, hemoglobinuria, leucopenia and relative lymphocytosis.

*Trichlorobenzene* in its toxicologic action is similar to the other chlorobenzene compounds. It is used chiefly as a solvent and in the manufacture of dye intermediates. It apparently is not a good insecticide and hence is not used in spraying or painting processes. Thus the danger of poisoning is lessened.

*Monochlorobenzene* is used in the manufacture of phenol, aniline, poisonous (war) gases, dyestuffs, etc. Its use as a solvent has not become general, hence there are no well authenticated cases of its toxic activities. Experimentally it

apparently produces similar but less marked symptoms to those of dichlorobenzene.

*Propylene dichloride:* Propylene dichloride is used as an ingredient in grain fumigants but its chief use is in dry cleaning fluids, generally with carbon tetrachloride or naphtha. The symptoms of poisoning are frequent in uninformed individuals, and may be the result of the carbon tetrachloride. Headache, vertigo, lacrimation, and irritation of the mucous membrane are the principal symptoms. Changes in the blood are those of marked anemia.

#### DISCUSSION

In practice it has been found that temperature and barometric variations affect the concentrations of the vapors in any establishment. Ventilation of quarters can now be controlled, although in practice it is much neglected and is the chief causative factor of poisoning. The effects of these vapors on the human body are also dependent upon other factors, such as the purity of the product, the admixture of several of the solvents, and the duration of exposure. Some of the liquids have specific actions on the central nervous system or the liver and kidneys, but practically all have an effect on the blood forming centers. The hematologic response may vary with the different poisons, and may show variations from the same poison under different conditions. It is not possible to establish a definite morphologic blood picture for each poison, but in general it may be stated that the compounds of the aliphatic series usually present a secondary anemia with or without a leucocyte response, and variations in the stippled cells, whereas those compounds derived from the aromatic ring generally affect a more serious blood damage manifested by types of aplastic anemia with leucopenic reactions.

The treatment of these conditions is as yet too uncertain to permit any suggestions except generalizations, i.e., removal of the cause, pure air, transfusions, blood regenerating substances (liver, iron, etc.).

The human body has been designed to live in an atmosphere of pure air. Its efficiency becomes immediately impaired when exposed to foreign material (gas) in the air. Certain substances have a higher toxicity than others. A general and safe precaution is to avoid any conditions under which the air breathed by



workmen becomes mixed with foreign material. It is therefore essential to ventilate adequately all quarters where these materials are used. A down-draft of fresh air should be furnished with spent air and solvent vapors drawn off at the floor level. When these solvents are used in deep or "closed" tanks, respirators may be worn, but it is always best to draw off the vapors from the bottom by means of a suction pump attached to a tube and to replace them with fresh air.

The danger of these solvents should be explained to every worker and they should be instructed in the preventive measures that have been instituted.

When these products are marketed for household use under trade names, etc., detailed instructions and precautions should be stated on each container, and it is especially important to state that the product must be employed only in a room with at least one window wide open. In this manner the housewife will employ proper precautions and be safe-guarded.

#### CONCLUSIONS

1. The dangers inherent in some of the newer commercial solvents which are finding wide-spread application in both industry and home are discussed.
2. Their importance as a possible factor in some of the blood dyscrasias is pointed out.
3. Their significance in legal medicine is noted.

#### *List of common solvents and the industries in which employed*

Methyl Acetate.....	Acetylcellulose, Nitrocellulose, Celluloid, Cellulose Ether, Resins, Oils, and Fats.
Ethyl Acetate.....	Acetylcellulose, Nitrocellulose, Oils, and Fats.
n-Propyl Acetate.....	Nitrocellulose, Celluloid, and Resins.
n-Butyl Acetate.....	Nitrocellulose, Celluloid, Cellulose Ether, Resins, and Oils.
i-Amyl Acetate.....	Nitrocellulose, Celluloid, Resins, Oils, and Fats.
Benzyl Acetate.....	Nitrocellulose.
Diethylphthalate.....	Nitrocellulose, Acetylcellulose, Resins. Otherwise as a softener.
Methyl Glycol Acetate.....	Nitrocellulose, Celluloid, Resins, Oils, and Fats.

Acetone.....	Acetylcellulose, Nitrocellulose, Celluloid, Resins, Oils, and Fats.
Ethylene Glycol.....	Dyes. As supplementary agent for lacquers.
Acetylene Tetrabromide....	Separation of Ores, Oils, and Waxes.
Acetylene Tetrachloride.....	Manufacture of Imitation Pearls. Waxes, Lacquers, Dye Intermediates, Gums, Camphor, Phosphorus, etc. Cellulose acetate.
Carbon Bisulphide.....	Fumigation, Rubber Industry, Rayon Industry, Sulphur, Resins, Wax.
Carbon Tetrachloride.....	Chemical Industry, Rubber, Fumigation, Cleaning and Dyeing.
Ethylene Chlorbromide.....	Chemical Industry.
Ethylene Dibromide.....	Water proofing preparations, "Ethyl" gasoline, Dye Intermediates.
Ethylene Dichloride.....	Fumigation, general solvent in Pharmaceutical and Chemical Industry, Fats, Oils, Waxes, Dry Cleaning.
Trichlorethylene.....	Dry Cleaning, Oils, Greases.
Tetrachlorethylene.....	Dry Cleaning, Waxes, Resins, Oils.
Monochlorbenzene.....	Chemical Industry, Poisonous Gases, Sulphur Dyestuffs.
Orthodichlorbenzene.....	Metal Polish, Lacquers, Varnishes, Dye Intermediates, Dry Cleaning, Fumigation.
Trichlorbenzene.....	Dye Intermediates, Oils.
Propylene Dichloride.....	Dry Cleaning, Greases, Fumigation.

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## RAPID METHODS FOR PREPARING PARAFFIN SECTIONS OF TISSUES\*

RUTH C. WANSTROM

*From the Department of Pathology, University of Michigan, Ann Arbor, Michigan*

The methods used by early microscopists working in the fields of normal histology and embryology were adopted by pathologists. As a result, it was accepted that the adequate impregnation of a block of tissue with paraffin was a slow process requiring many days for its accomplishment. For the preparation of sections from delicate structures, with artefacts reduced to a minimum, prolonged time and careful grading of reagents are still essentials, but in practical work in histopathology this nicety of result may profitably be sacrificed in order to place the diagnosis in the hands of the surgeon or internist during the period of collection of objective data from other sources. Only by so doing can the pathologist be a participating consultant and not a referee in the practice of medicine.

The methods which are outlined in this paper are those which are in daily use in the Department of Pathology of the University of Michigan. They are not necessarily the best methods, but they have proved to be eminently practicable in the handling of a large material (17,000 surgical specimens per year) in such a manner as to reduce the demand for diagnoses from less satisfactory frozen sections to a low level. In spite of the large volume of material passing through the laboratory, less than two frozen section diagnoses a day, on the average, are requested. Analysis of these requests shows that many are inspired by clinical curiosity rather than by the necessity of the situation.

### ROUTINE OVERNIGHT PROCEDURE

Those pathologists and surgeons who are accustomed to an interval of from three to five days for the preparation of paraffin

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sections will consider a routine method which gives an overnight service a "rapid" method. For this reason, and since it forms the basis of still more rapid procedures, the routine method in use must be outlined.

The successful preparation of paraffin sections within twelve hours, or on an overnight schedule, permitting all specimens received before five o'clock in the afternoon to be ready for diagnosis at ten o'clock the next morning, offers no difficulty. The material is placed in 10 per cent formalin in the operating rooms and sent to the laboratory during the day. The portions for sectioning are selected between three and five o'clock in the afternoon. By that time the smaller specimens have an adequate formol fixation. If fixation is imperfect in blocks selected from large specimens, the process is completed in the first alcohol. As selected, the tissue blocks are put into 96 per cent alcohol. (Actually, filtered, once-used, absolute alcohol is utilized at this step in the interest of economy.) Two to four changes of absolute alcohol, two of xylol and two of paraffin follow in sequence. When the tissue blocks from a case are large and numerous, wide-mouthed 4 ounce bottles are used, of the type called pomade bottles. For smaller specimens, 2 ounce and 1 ounce bottles are employed, and many biopsy specimens are handled in small straight vials which are always placed in larger wide-mouthed bottles to prevent overturning. Not only is economy effected by using bottles of varying sizes, but also the number of changes of absolute alcohol may be varied, being safely reduced to two when the material is small in amount or finely divided. The time in xylol should not be extended beyond that necessary to make the tissue translucent. A longer period in xylol renders the tissues brittle and makes the cutting of thin sections more difficult. Gentle blotting of the tissues between each two steps preceding placing in paraffin conserves reagents and expedites penetration by the solvents. The changes in this series are carried out on such a schedule that the second paraffin is reached at 11:00 p.m. In this, the tissues remain in the oven, with corks removed from the bottles, until 7:00 a.m., when they are blocked in fresh paraffin.

*The routine paraffin method*

1. 10 per cent formalin, 1-12 hours (depending on time of operation).
2. 96 per cent alcohol (or filtered, used absolute alcohol).
3. Absolute alcohol, 2-4 changes in 1-5 hours.
4. Xylol I,  $\frac{1}{2}$ -1 hour.
5. Xylol II,  $\frac{1}{2}$ -2 hours.
6. Paraffin I, 1 hour.
7. Paraffin II, 6 hours, or overnight.

## INTERMEDIATE PARAFFIN METHODS

When circumstances do not permit the inclusion of material in the routine, overnight run, because the diagnosis is desired on the same day, practically any non-calcareous tissue can be embedded in paraffin in two or more hours (See fig. 1). Cutting the blocks of tissue thinner than the usual 8 mm., carrying out

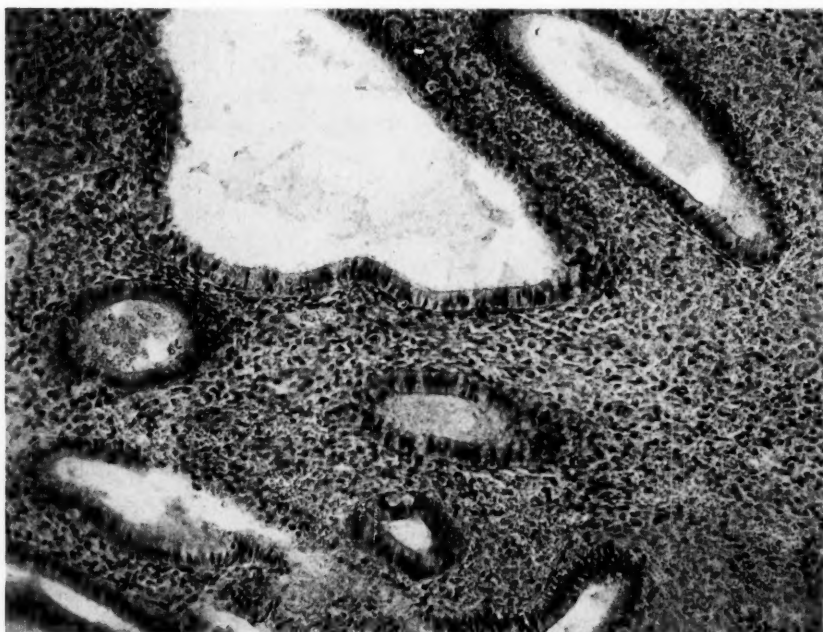


FIG. 1. POLYPOID GLANDULAR AND INTERSTITIAL HYPERPLASIA OF THE ENDOMETRIUM

Tissue ready for sectioning in one and one-half hours after receipt. Acetone dehydration. Hemalum and eosin stain.  $\times 138$ .

the processes of fixation and dehydration in the oven, or utilizing the triple abilities of water-free acetone in fixation, dehydration and solution of paraffin, are devices for facilitating the process. Ten minutes is sufficient time for the final blocking, sectioning, plating in the celloidin sheet<sup>1</sup> and staining with hemalum and eosin. No further reference is made to these cutting and staining operations in the sample procedures outlined here.

*Intermediate paraffin methods*

- A. 1. 10 per cent formol, in oven, 15-30 minutes.  
2. Absolute alcohol, 4 changes, in oven; total time, 1-1½ hours.  
3. Xylol I, in oven, 20 minutes.  
4. Xylol II, in oven, 40 minutes.  
5. Paraffin I, 20 minutes.  
6. Paraffin II, 40 minutes.

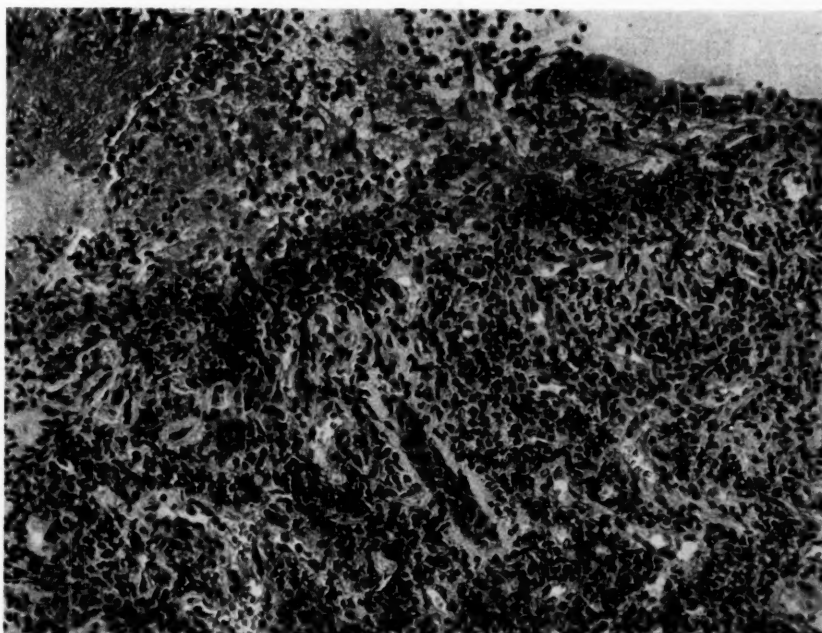


FIG. 2. 2199-AO. ULCERATING VASCULAR PYOGENIC GRANULOMA OF THE NASAL MUCOSA

From a piece of tissue 7 mm. in diameter, ready for sectioning in two and one-half hours. Alcohol dehydration. Hemalum and eosin stain.  $\times 200$ .

- B. 1. 10 per cent formol, 15-30 minutes.  
2. Water-free acetone, 2 changes in 20-40 minutes.  
3. Paraffin I, 20 minutes.  
4. Paraffin II, 30 minutes.

Actual working schedules as applied to particular specimens may be of interest and value.



Specimen 2199-AO was a biopsy of soft tissue from the turbinate region (See fig. 2.) Paraffin blocks were ready for sectioning in two hours and five minutes after receipt of the tissue.

1. 10 per cent formol, 15 minutes.
2. Absolute alcohol, 3 changes in 40 minutes.
3. Xylol, 2 changes in 30 minutes.
4. Paraffin, 2 changes in 40 minutes.

Tissue specimens from the cervix, 2200-AO, were ready for sectioning from paraffin in two hours and fifty-five minutes.

1. Formol, 15 minutes.
2. Absolute alcohol, 3 changes in 55 minutes.
3. Xylol, 2 changes in 40 minutes.
4. Paraffin, 2 changes in 45 minutes.

#### RAPID PARAFFIN METHOD

By a rapid paraffin method is understood a procedure by which tissues are made ready for sectioning in paraffin in approximately one hour, or less. (See figs. 3 and 4.) This method lends itself particularly to the handling of endometrial curettings, specimens obtained by transurethral prostatic resection, and small biopsies in general. These are the very specimens in which the mechanical difficulties and the risks in the preparation of frozen sections are the most serious. If very thin blocks, less than two millimeters thick, are cut, large specimens may be examined similarly.

The rapid method is simply a compression of those outlined above, taking advantage of every technical device which will facilitate dehydration and infiltration. The volume of reagent used should be at least fifty times that of the tissue. The entire process is carried out in the oven.

#### *Rapid paraffin method*

1. 10 per cent formalin, during transportation from Surgery.
2. Absolute alcohol, in oven, 2 changes in 20 minutes.
3. Xylol, in oven, 2 changes in 20 minutes.
4. Paraffin, in oven, 2 changes in 20 minutes.

For the second xylol a 50 per cent solution of paraffin in xylol is sometimes used. This probably facilitates impregnation, but is not essential.

If both clinicians and pathologists are aware that thin sections, of such good quality that they can be photographed if desired, can be prepared by paraffin method in from one to three hours,



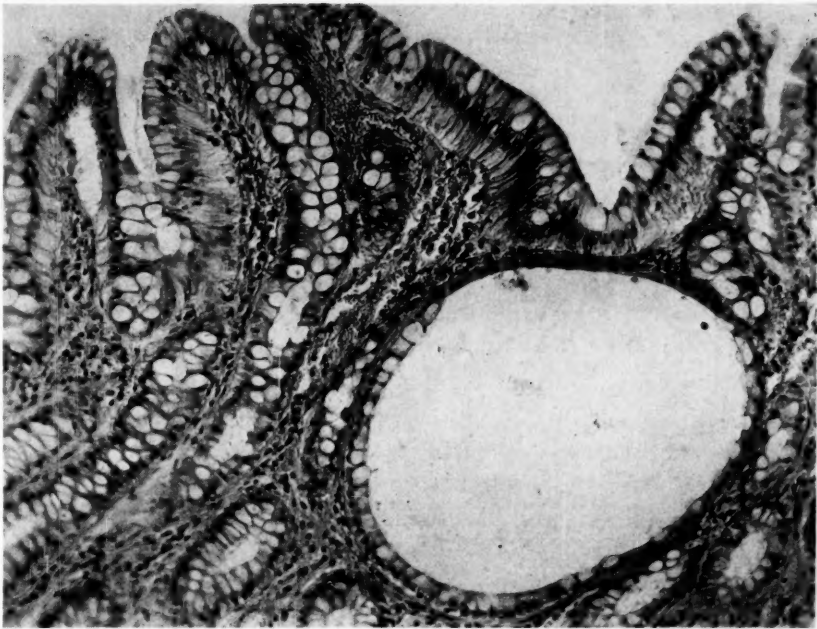


FIG. 3. BENIGN POLYPOID HYPERPLASIA OF THE RECTAL MUCOSA  
Tissue infiltrated with paraffin and ready for sectioning one hour after being received. Alcohol dehydration. Hemalum and eosin stain.  $\times 140$ .

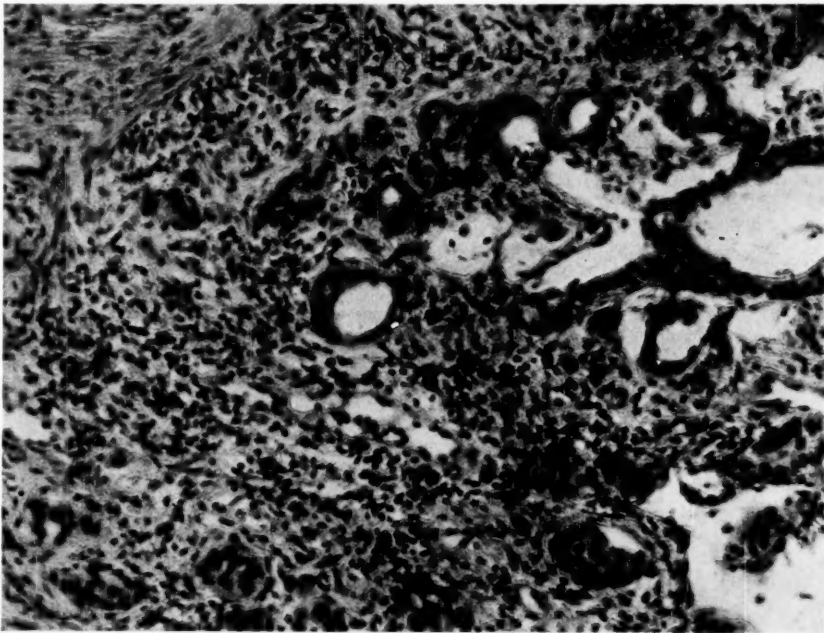


FIG. 4. ADENOCARCINOMA OF THE RECTUM WITH METAPLASIA TO SQUAMOUS CELLED TYPE

Prepared in one hour after receipt. Alcohol dehydration. Hemalum and eosin stain.  $\times 140$ .

depending upon the size and consistency of the specimen, the need for diagnoses from frozen sections is largely removed. Frozen sections are indispensable for the study of lipids; for the immediate identification of structure, as in the search for parathyroids, and occasionally for the identification of lesions found unexpectedly in the course of surgical operations. In general, however, frozen sections are inferior in quality, and yield less secure diagnoses which are usually made under such a stress that the pathologist is not at his best. Multiple small fragments of tissue seldom can be adequately examined in frozen sections, and pathologists should decline to assume responsibility for diagnoses based upon inadequate sampling of the available material, as is often the case when curettings are examined by this method. It is difficult to conceive a situation in which a delay of one hour in making a diagnosis upon endometrial curettings would interfere with the proper clinical management of the case.

It still remains true in many hospital laboratories that poor, or even hopelessly bad, tissue technic is the chief reason for incorrect or uncertain pathological diagnoses.

#### REFERENCE

- (1) WARTHIN, A. S.: The molasses plate method for the staining of many paraffin sections at one time. *Jour. Lab. and Clin. Med.*, **9**: 554-561. 1924.

## QUANTITATIVE ISOLATION OF ETHYL ALCOHOL FROM TISSUES OF ALCOHOLICS\*

ALEXANDER O. GETTLER AND HENRY SIEGEL

*From the Chemical Laboratories of the Chief Medical Examiner's Office and of  
Washington Square College, New York University, New York City*

Much interest has been shown and many papers have been published during the past few years on the quantitative determination of ethyl alcohol in body fluids, tissues, and expired air. Investigators in this field make use of the alcoholic content of the aforementioned materials in deciding whether individuals involved in vocational or motor accidents were intoxicated at the time or not.

Most all of the published quantitative methods for ethyl alcohol depend on one of two chemical reactions: (a) The tissues are distilled with steam, the alcohol in the distillate is oxidized to acetic acid under carefully regulated conditions and the resulting acidity is titrated with standard alkali. From the titration figure the amount of alcohol is calculated. (b) The alcohol in the samples of body materials is distilled, aeriated, or absorbed into a standard sulfuric-dichromate oxidizing mixture. The alcohol reduces its equivalent of dichromate and the amount of reduction is determined colorimetrically, or by titrating the quantity of dichromate that is left. From the degree of reduction the alcohol content is calculated.

It is obvious that the two reactions above, upon which practically all of the quantitative methods for alcohol depend, are not specific tests for ethyl alcohol for many volatile organic substances behave very much like alcohol toward the reagents used in these quantitative methods. As examples of such substances some-

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times found in tissues, we need only mention acetone, acetaldehyde and paraldehyde. With this in mind, picture the analyst on the witness stand, testifying that he found a certain quantity of alcohol in the brain of the deceased. What answer could he give, were the lawyer to ask him, "how do you know that that which you quantitatively determined was ethyl alcohol? What specific tests for ethyl alcohol have you performed?" No proper answer could be given to these questions. The following method was devised to meet just such an emergency.

#### METHOD IN DETAIL

##### *The distillation*

As soon as the tissue is removed from the body it is placed in a receptacle, sealed, and refrigerated. When ice-cold, about 200 grams are finely hashed, of which 150 grams are weighed out and placed in a 500 cc. distilling flask. All these operations should be done quickly in order to avoid any appreciable loss of alcohol by volatilization. Two hundred cubic centimeters of water and 0.5 cc. of white mineral oil are now added and the flask and contents are set up for steam distillation. The receiving flask is packed in ice. Distillation is continued until about 200 cc. of distillate have collected. Experiments have shown that practically all of the alcohol that was present in the tissues is recovered in this 200 cc. distillate. The presence or absence of alcohol in the distillate can rapidly be established by oxidizing about 2 cc. of the distillate with a red hot copper spiral, and then testing the oxidized and cooled portion with Schiff's aldehyde reagent.

##### *The quantitative isolation*

The entire *steam distillate* is quantitatively transferred to the *rectification flask* (fig. 1, *F*) and about 2 grams of granulated zinc are added. While the flask is immersed in ice water and its contents continually rotated, 300 grams of anhydrous  $K_2CO_3$  are slowly added. The solution should not become warm. The rectification arm (*K*) is attached to a flask (*F*) by a ground glass joint (*J*) and the whole apparatus set up for rectification as shown in figure 1. The arm is previously cleaned by boiling concentrated nitric acid in the flask, then thoroughly washed with water and dried. The rectification flask should rest on an asbestos-centered wire gauze and be heated with a micro burner. The flame is so regulated that the solution should boil 15 to 25 minutes without permitting the hot vapors to rise beyond (*K*). The flame is then increased so that the alcoholic ring of condensate rises from (*K*) to (*L*) in 3 to 5 minutes. The height of the ring is indicated by a visible ring of condensate, or by running the hand along the arm and determining the height of the ring by the temperature.

Heating is continued at this same rate. If the rate of heating is correct, then the bend (*L*) should feel warm or somewhat hot ( $78^{\circ}\text{C}.$ ) to the hand. This indicates that alcohol is distilling (although no steam has reached the bend) into the calibrated receiving tube (*N*) immersed in solid  $\text{CO}_2$ -acetone mixture (*O*).

After the alcohol has distilled, steam will gradually follow, condense, freeze, and clog up the tube below (*M*), and therefore, as the heating is continued, back pressure will force the solution up the safety tube (*I*). This marks the endpoint. The time, from the rise of the first ring of alcohol condensate to the

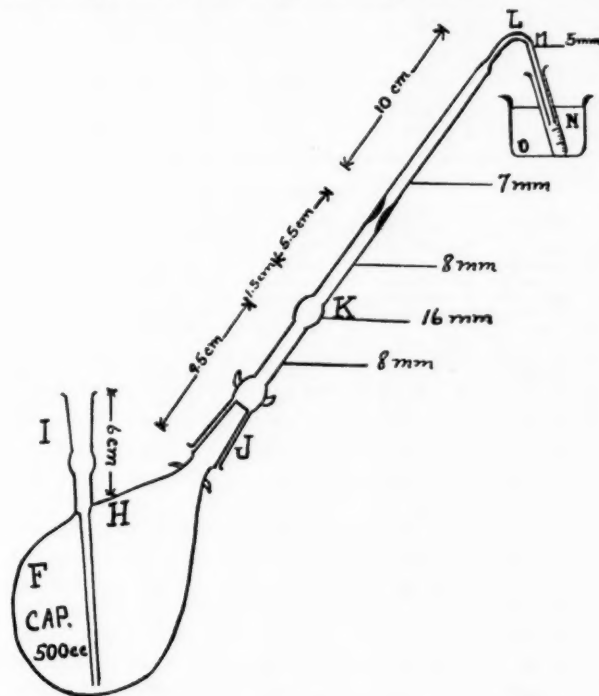


FIG. 1

endpoint, for large amounts of alcohol is about 25 minutes. If the rate of heating is too slow, then the water will not come over but merely condense and drop back, the arm acting as an air-cooled reflux. In that case, if the actual distillation (from rise of ring to endpoint) is not complete after about 30 minutes, increase the flame slightly. If the rate of heating is too fast much water will come over before the endpoint is reached. This is indicated by (1) the bend (*L*) reaches temperature of steam; (2) drops of water are seen to form just beyond the bend, yet the opening does not become clogged; (3) violent boiling of the solution; (4) uneven wetting of the sides of the arm. If this occurs then

decrease the size of the flame. With a little experience this rarely occurs. It is best to have the flame too small rather than too large. It is very important

TABLE 1  
RECOVERY OF ETHYL ALCOHOL FROM PURE SOLUTION

GRAMS EtOH ADDED TO 20 CC. WATER	GRAMS EtOH RECOVERED	PER CENT YIELD	GRAMS LOST OR GAINED	PER CENT LOSS OR GAIN
0.192	0.184	96	-0.008	-0.005
0.307	0.299	97	-0.008	-0.006
0.384	0.373	97	-0.011	-0.007
0.461	0.444	96	-0.017	-0.011
0.576	0.582	101	+0.006	+0.004
0.768	0.759	99	-0.009	-0.006
0.979	0.950	97	-0.029	-0.020

TABLE 2  
RECOVERY OF ADDED ETHYL ALCOHOL FROM TISSUES

	GRAMS ALCOHOL ADDED TO 150 GRAMS TISSUE	GRAMS ALCOHOL RECOVERED	ADDED ALCOHOL IN PER CENT OF TISSUE WEIGHT	ALCOHOL RECOVERED IN PER CENT OF TISSUE WEIGHT	PER CENT ALCOHOL GAIN OR LOSS	PER CENT ALCOHOL RECOVERED
1	0.0785	0.0690	0.052	0.046	-0.006	87.9
2	0.1570	0.1380	0.105	0.092	-0.013	87.9
3	0.2336	0.1993	0.156	0.133	-0.023	85.3
4	0.3114	0.2912	0.208	0.194	-0.014	93.2
5	0.3126	0.2836	0.208	0.189	-0.019	90.7
6	0.3893	0.3832	0.259	0.255	-0.004	98.4
7	0.3918	0.3765	0.261	0.251	-0.010	96.1
8	0.4671	0.4598	0.311	0.307	-0.004	98.4
9	0.4671	0.4675	0.311	0.312	+0.001	100.1
10	0.4709	0.4598	0.314	0.307	-0.007	97.6
11	0.5450	0.5365	0.363	0.358	-0.005	98.4
12	0.5839	0.5901	0.389	0.393	+0.004	101.1
13	0.6278	0.6131	0.419	0.409	-0.010	97.6
14	0.7007	0.6821	0.467	0.455	-0.012	98.7
15	0.7785	0.7587	0.519	0.506	-0.013	97.4
16	0.7848	0.8047	0.523	0.536	+0.013	102.5
17	0.8564	0.8584	0.571	0.572	+0.001	100.2
18	0.9794	0.9503	0.653	0.634	-0.019	97.0

that the receiving tube should be immersed in the cooling bath as deeply as possible.

As soon as the endpoint is reached (as evidenced by the solution flowing out of the safety tube (*I*)), the arm (*K*) is lifted out of the receiver (*N*). The



TABLE 3  
COMPARISON OF QUANTITATIVE RESULTS OBTAINED WITH ISOLATION METHOD AND  
WITH OXIDATION METHOD

CASE	ISOLATION METHOD— PER CENT ALCOHOL FOUND IN BRAIN	OXIDATION METHOD <sup>1</sup> — PER CENT ALCOHOL FOUND IN BRAIN	DIFFERENCE IN PER CENT ALCOHOL BY THE TWO METHODS
1	0.11	0.12	-0.01
2	0.07	0.07	0.00
3	0.29	0.30	-0.01
4	0.20	0.19	+0.01
5	0.25	0.26	-0.01
6	0.20	0.19	+0.01
7	0.12	0.13	-0.01
8	0.06	0.06	0.00
9	0.15	0.17	-0.02
10	0.16	0.18	-0.02
11	0.31	0.33	-0.02
12	0.00	0.00	0.00
13	0.37	0.41	-0.04
14	0.37	0.41	-0.04
15	0.29	0.32	-0.03
16	0.43	0.49	-0.06

<sup>1</sup> Gettler, A. O. and Tiber, A.: The quantitative determination of ethyl alcohol in human tissues. Arch. Path. and Lab. Med., 3: 75. 1927.

TABLE 4  
MICRO-BOILING-POINT DETERMINATIONS

Anhydrous ethyl alcohol, kept over $\text{CuSO}_4$ .....	78.1
Anhydrous ethyl alcohol, kept over saturated solution of $\text{K}_2\text{CO}_3$ .....	78.2
Anhydrous ethyl alcohol, kept over saturated solution of $\text{K}_2\text{CO}_3$ .....	77.9
Anhydrous ethyl alcohol, kept over saturated solution of $\text{K}_2\text{CO}_3$ .....	78.1
Ethyl alcohol isolated from tissues, Case 1.....	78.1
Ethyl alcohol isolated from tissues, Case 2.....	78.8
Ethyl alcohol isolated from tissues, Case 3.....	78.3
Ethyl alcohol isolated from tissues, Case 4.....	78.5
Ethyl alcohol isolated from tissues, Case 5.....	77.5
Ethyl alcohol isolated from tissues, Case 6.....	77.8
Ethyl alcohol isolated from tissues, Case 7.....	78.0
Ethyl alcohol isolated from tissues, Case 8.....	78.2
Ethyl alcohol isolated from tissues, Case 9.....	79.0
Ethyl alcohol isolated from tissues, Case 10.....	77.8
Ethyl alcohol isolated from tissues, Case 11.....	78.7
Ethyl alcohol isolated from tissues, Case 12.....	78.5



receiving tube containing the isolated alcohol is removed from the cooling bath and kept at room temperature until any ice that may be present has melted, after which it is placed in a bath of ice water. Three-tenths cubic centimeter of saturated aqueous  $K_2CO_3$  solution and some crystals of anhydrous  $K_2CO_3$  are added to the alcohol in the tube and thoroughly mixed for a few minutes with a fine glass rod. If much water has distilled over, solid  $K_2CO_3$  only is added and then stirred for a longer time, about 10 minutes. Centrifuge the mixture until a clear upper layer is obtained (about 1 minute). Let stand in ice water about 3 minutes. Read the volume of the alcohol (upper layer). Excess solid  $K_2CO_3$  must always be present. Under these conditions ( $0^\circ$  temperature and in contact with solid  $K_2CO_3$ ) according to Seidell ("Solubilities of organic compounds") the alcohol layer will be of the following constant composition:

	<i>per cent</i>
Ethyl alcohol.....	91.9
$K_2CO_3$ .....	0.04
Water.....	8.1

1 cc. of this (constant composition) layer at  $0^\circ C$ . weighs 0.833 gram.

*Calculation:*

1 cc. of alcohol layer contains  $0.833 \times 0.919 = 0.7655$  gram alcohol  
 $0.7665 \times$  cc. of upper layer = grams of EtOH in 150 grams tissue  
 or  
 $0.5103 \times$  cc. of upper layer = percentage EtOH in tissue.

Tables 1 to 3 indicate the results obtained with the method described.

#### THE IDENTIFICATION OF THE ISOLATED ALCOHOL

##### *I. By micro boiling-point determination*

(a) The boiling point of the isolated liquid may be directly determined without any further purification, provided acetone or acetaldehyde (paraldehyde) are not present in appreciable amounts. Emich's Micro boiling-point method, as described below was used. Table 4 gives the boiling points that were obtained on the ethyl alcohol isolated from human tissues.

The results charted in table 4 show that the boiling point of the (constant composition) isolated alcohol layer, is quite close to that of pure ethyl alcohol.

(b) If it is desired to obtain 100 per cent pure ethyl alcohol from the isolated portion, before attempting the boiling point determination, the following technique is recommended:

*The micro drying of the isolated alcohol (fig. 2)*

By means of a capillary tube, two drops (or more, if available) of the alcohol layer is transferred into a larger capillary tube (A) one end of which was formed

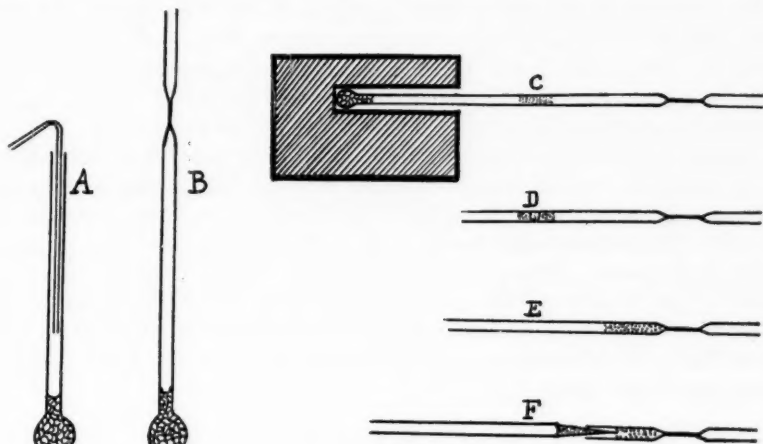


FIG. 2

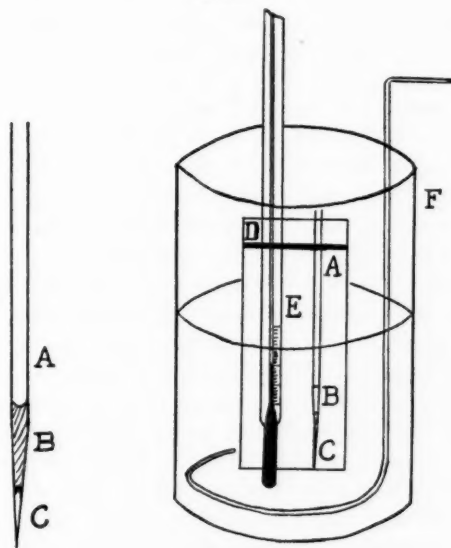


FIG. 3

into a little bulb, the bulb being previously filled with finely granulated calcium oxide (or anhydrous copper sulphate). The volume of the calcium oxide must be larger than the volume of the alcohol introduced. The capillary is then

centrifuged, in order to force all the liquid into the bulb. The open end is then sealed (*B*) and placed into a boiling water bath for 5 minutes. The capillary is now again centrifuged, to bring all the liquid into contact with the calcium oxide, and allowed to stand over night at room temperature. The bulb end of the sealed capillary (*C*) is now placed into the cavity of an aluminum heating block and the temperature of the block is raised to 95°C. and kept so for five minutes. The alcohol in the bulb portion distills and appears as a condensate in the cold part of the capillary outside of the heating block (*C*). The capillary is now removed from the block, and cut in two at the middle (*D*). The part containing the condensate is centrifuged in order to force the alcohol to the sealed end (*E*). Experiments have shown that by this treatment, anhydrous ethyl alcohol is obtained. A small drop is taken up by a 1 mm. bore capillary tube (*F*) which is then sealed by bringing the tip of it into the edge of a Bunsen flame for a second. The walls of the capillary being extremely thin, almost instantly fuse together. Sealing in this way always leaves a bubble of gas (air) in the tip of the capillary, between the sealed portion of glass and the drop liquid (fig. 3, *C*), which serves to prevent superheating of the liquid during the boiling point determination.

#### MICRO-BOILING-POINT DETERMINATION

The capillary containing the drop of liquid is then set up in an ordinary melting point apparatus (fig. 3) (beaker, liquid bath, thermometer and stirrer), and the temperature of the bath raised slowly and regularly. According to F. Emich the true boiling point of a pure liquid is the temperature at which the droplet in the capillary reaches the surface of the bath. This is true, however, only if the gas bubble in the tip of the capillary is so small that the effect of its expansion is negligible, and also if the temperature is raised regularly and uniformly throughout the entire bath. If the little drop in the capillary is pure anhydrous alcohol, it should start ascending at about 76°C. and should reach the surface of the bath between 77.5° and 78°C.

If the boiling point is found to be much lower (66°–76°) than that of ethyl alcohol, it indicates the admixture of lower boiling point liquids such as acetone or acetaldehyde (paraldehyde). Appreciable amounts of acetone are only met with in severe diabetics. Large amounts of acetaldehyde are only found if paraldehyde had been administered within 12 to 18 hours before death. The presence of either of these substances can be readily demonstrated by the ordinary qualitative tests. Should either of these substances be present in appreciable amounts, it is necessary to remove them before the alcohol is measured and identified. This procedure is beyond the scope of the present paper, but will be described in a future publication.

For further identification of the isolated alcohol we recommend:

- II. *Molecular Weight Determination.* J. B. Niederl and Wm. J. Saschek, *Mikrochemie*, XI, 237, 1932.

III. *Carbon and Hydrogen Determination.* J. B. Niederl and R. T. Roth, Ind. Eng. Chem., Analyt. Ed., 6, p. 272, 1934.

IV. *Making Ethyl Benzoate Derivative.* Gettler, Niederl and Benedetti-Pichler, Mikrochemie, 1932, vol. XI, p. 184.

#### SUMMARY

1. A quantitative method for ethyl alcohol in tissues is described. The method is based on the actual isolation of the alcohol from the tissues, and the measurement of its volume.

2. The isolated alcohol is then identified by boiling point, molecular weight, carbon and hydrogen analysis, and the making of the ethyl benzoate derivative.

3. The possibility of definitely identifying the isolated ethyl alcohol is a feature that is lacking in all other quantitative methods for alcohol.

## NEWS AND NOTICES

The annual convention of The American Society of Clinical Pathologists for 1937 will be held in Philadelphia June 3, 4.

Arrangements are in progress for this meeting and also for the Tumor Seminar which will be conducted in conjunction with it.

The marriage of Anna May Young, M.D. and Edmund Earl Beard, M.D. took place on August 29th, 1936.

At the Tumor Institute conducted by the University of Illinois the American Society of Clinical Pathologists was well represented, not only in the audience of six hundred who attended, but also on the program.

Stanley Reimann, M.D., of Philadelphia, presented an interesting and able discussion of "The Biology of The Cancer Cell" in which he clearly outlined the future rôle of the pathologist and, incidentally, the future educational requirements necessary to meet his responsibilities.

A paper of great interest was that presented by James Ewing, M.D. of New York emphasizing the limitations of the biopsy, particularly the puncture biopsy, in which the possible errors of this procedure were pointed out.

Dr. Ewing also called attention to the need of more specialization in tumor diagnosis and suggested that those interested in this field could well proceed by studying the material now collected by the various tumor registries.

Among those attending a Conference of Southern Pathologists held in Washington on November 16th were Drs. Kracke, Giordano, Keilty, Norris, Ikeda, Larson, Neal and Kilduffe. At the conclusion of the conference, those attending were the guests of Georgetown University at luncheon and then visited various places of interest. Assembling later at the home of Col. E. C.

Whitmore, local host to the conference, the party was later tendered a dinner at the Army and Navy Club after which they attended the ceremonies marking the one hundredth anniversary of the Army Medical Museum and Library.

At the recent Southern Medical Convention held in Baltimore November 17th to 21st the American Society of Clinical Pathologists was well represented.

Among those in attendance were Drs. Lamb, Ikeda, Larson, Foord, Hartman, Simpson, Kilduffe, Giordano, Magath, Sanford, Thomas, Konzelman, Neal, and Kracke.

The following appeared upon the program: R. A. Vonderlehr, W. M. Simpson, A. H. Sanford, F. C. Helwig, M. P. Neal, and K. M. Lynch.

Among those showing Scientific Exhibits were R. R. Kracke, R. A. Vonderlehr, R. D'Annoy and E. Von Haam, and the Board of Registry of The A. S. C. P.

The first meeting of the American Board of Pathology, held during the Southern Medical Convention in Baltimore, was attended by a large number of candidates for certification.

The next meeting of the Board will be held in Chicago on March 26th and 27th in connection with the meeting of the American Association of Pathologists and Bacteriologists, and will be followed by a meeting in Philadelphia in conjunction with the 16th Annual Convention of the Society.

## EDITORIALS

### THE FROZEN SECTION FETISH

The buzzer rings. The pathologist leaps to his feet, hastily excuses himself from his consultation with a member of the medical staff, and runs to the operating room. Seated on the amphitheater benches are a dozen visiting physicians to whom the surgeon has just described the hard fixed nodule in the breast of the woman who is asleep on the operating table. Greeting the panting pathologist with a patronizing smile, he turns to the audience: "Here is our pathologist! He will have the diagnosis for us in three minutes!" Like a trained seal, the pathologist catches the small bit of tissue and rushes back to his laboratory. A few deft motions and the stained section is placed on the stage of the microscope. Realizing that the surgeon and his expectant audience await the diagnosis within three minutes, the pathologist hastily glances through the microscope and rushes back to the operating room. "Scirrhou carcinoma!" he gasps, and withdraws as the surgeon undertakes the radical operation.

In the instance just cited, drawn from an actual observation, the diagnosis required no great skill on the part of either the surgeon or the pathologist, because it was quite apparent from the history, physical findings, and the gross inspection of the bit of tissue that it was scirrhou carcinoma. The microscopic observations only confirmed what the pathologist already knew. The gross appearance of the well-developed scirrhou carcinoma of the breast was so characteristic that it should have been recognized as such by the surgeon.

In contrast to the situation cited, there are instances in which doubt exists in the mind of the pathologist after he has examined the frozen section. Two new developments contribute to the advent of a new era in the rapid section diagnosis of biopsy material. The first is that women have become more conscious



of the significance of a nodule in the breast, with the result that the earliest manifestations of the cancerous lesions of the breast, and other organs, are now being seen by the pathologist. As a result, more "borderline" lesions are encountered. The second factor contributing to the new era in rapid section diagnosis is the more adequate training in gross pathology of the young surgeon who has devoted three to five years to special training in surgery before undertaking independent work in this field.

The method of freezing tissue preparatory to cutting and staining it was introduced in this country just before the dawn of the twentieth century. Certain coincidental developments in American surgery led to the popularity of the frozen section method. The field of surgery was expanding by leaps and bounds. Greater possibilities for the cure of malignant disease by surgery were visualized. The importance of the early diagnosis of cancer was emphasized and anything which would hasten a diagnosis was welcomed. As a result, the frozen section method was eagerly seized upon because the diagnosis could be made while the patient was still on the operating table.

Campaigns were launched to teach the lay public some of the facts concerning cancer. In the beginning, particular emphasis was placed upon cancer of the breast, largely because of its surgical accessibility and because of the knowledge that some of these lesions could be cured surgically if attacked early enough. At the same time there was an active discussion as to whether chronic cystic mastitis was inflammatory or neoplastic in nature. Bloodgood was a pioneer in these commendable efforts. He demonstrated that so-called "chronic cystic mastitis" was usually a benign lesion and stated that in doubtful cases the diagnosis could be made only at the operating table by means of the frozen section. There then arose the feeling that cancer is always characterized by rapid growth and must be treated immediately after the diagnosis is made. From this background developed the surgical pattern as we now observe it in most hospitals, with such great emphasis and reliance upon the immediate frozen section.

While surgery has made enormous strides since the time of the barber-surgeons, largely due to the contributions of a dentist

(Morton) and a chemist (Pasteur), it is still essentially an art. While modern developments have removed much of the spectacle from the practice of surgery it is still a more or less spectacular art. It was natural that anything which would add to the drama of the spectacle would be welcomed and certainly the addition of the frozen section provided a distinctly dramatic touch to the performance. What better impression can the surgeon make upon visiting practitioners, or on one unacquainted with operating room routine, than to toss a specimen to a waiting pathologist and await his return a few minutes later, often out of breath, to give the diagnosis. The sooner he returns with the diagnosis the better is the impression made on the bystanders and the better pleased is the surgeon. The result is that every device is used to reduce the time necessary to freeze, cut, stain, and examine the tissue. The pathologist senses the pressure of the surgeon's impatience, the fact that a patient is waiting on the operating table, usually anesthetized, and the end result is a section poorly cut and stained and inadequately studied. Time does not permit the cutting of several sections from carefully selected areas of the suspected tumor. No distinction is made between the operating room diagnosis that is corroborative and the final diagnosis by the pathologist, which is really diagnostic.

In most instances the entire dramatic procedure is unnecessary. If the surgeon has faith in his pre-operative diagnosis and his gross observations at the time of the operation, such a practice is unnecessary. If he feels that frozen section diagnosis is required then the case is usually a borderline one. In such a case the welfare of the patient is much better served by the removal of the suspected lesion with a wide border of normal tissue, followed by the preparation of a series of permanent sections. The development of new rapid methods for the preparation of excellent permanent sections removes many of the reasons for the making of frozen sections. Well-fixed and well-stained permanent sections can be made and studied adequately in from twenty-four to forty-eight hours after the time of operation. It is, in fact, possible to prepare permanent sections from small pieces of tissue, such as uterine curettings, in two hours. If the surgeon is in

doubt following the gross examination of the tissue then, in fairness to the patient and to the pathologist, he should content himself with having made the biopsy, send the patient to his room, and wait until several well-prepared sections may be studied.

Objection to this practice might be raised when the lesion is intra-abdominal, in that such a procedure would require a second laparotomy. Assuming that every necessary diagnostic procedure has been carried out prior to the laparotomy such a possibility should not arise. If cancer is present it can usually be recognized in the gross and the decision then made as to whether or not the radical operation should be done. An occasional source of doubt may be the gastric ulcer in which carcinoma may have developed. In such an instance the approved method of treatment is resection of the ulcer with a wide zone of adjacent healthy tissue, so that it really makes little difference. Carcinoma of the head of the pancreas cannot ordinarily be resected and a piece of tissue is taken only for corroboration of the diagnosis.

In tumors of the breast, excision of the entire lesion with a wide margin of adjacent normal tissue does not traumatize the suspected area and, hence, does no damage for cancer ordinarily is not a fulminating disease. When it is, it is unmistakably cancer at the time the patient comes to operation and a frozen section is not necessary. The average duration of cancer of the breast when seen by the surgeon for the first time has been about fourteen months; at least a palpable lump has been present for that length of time. A delay of two days, then, when no trauma has been done to a doubtful mass in the breast, should not accelerate the progress of the disease.

There are certain hazards which must be recognized in the frozen section method of rapid tissue diagnosis. Frequently the surgeon excises a single piece of tissue which appears to him to contain the suspected lesion, but the pathologist finds no evidence of carcinoma in the small excised piece. It may then be assumed that cancer is not present, whereas the careful examination of many permanent sections will often reveal the presence of cancer in other areas. Furthermore, even when frozen sections are made, most tissue pathologists do not feel secure until they have

studied many permanent sections derived from the same material. Frequently it is not possible to make permanent sections because all of the material is utilized for the frozen sections. The limitations of the frozen section method when applied to small pieces of soft friable tissue, such as lymphnodes and uterine curettings, are too well recognized to require further comment. The prevalent use of high-frequency electrical cutting currents further complicates the problem for the pathologist who places his dependence upon the frozen section as the intense heat generated by the electric knife or loop often produces bizarre changes in the tissues, which are much more difficult to interpret in frozen sections.

In a few of the larger clinics certain pathologists have acquired great skill in the preparation of frozen sections. In many laboratories, however, the frozen section is poorly prepared and an accurate diagnosis is often not possible. *Any diagnostic method which yields good results only in the hands of a few experts is not adaptable to the "average" hospital laboratory.* The welfare of the patient and pathologist would be much better served if reliance were placed upon several well-prepared permanent sections.

During the past few years several ingenious methods for obtaining biopsy material have come into vogue. Their use requires even greater ingenuity on the part of the pathologist to avoid error in determining an accurate diagnosis. Here again, the pathologist is much less handicapped by the study of permanent sections. The limitations of the so-called "needle biopsy" and "punch biopsy" are apparent to every tissue pathologist of broad experience. Simple excision, followed by the examination of permanent sections made from all of the suspected tissue, is much more apt to yield the desired information.

Ewing, whom all pathologists revere as a master tissue diagnostician, was one of the first to point out the dangers and the limitations of the frozen section diagnostic method. In 1925, Ewing<sup>1</sup> wrote: "Having made more errors by the frozen section method in breast cases than by the gross examination, I have not resorted to frozen sections in this field for many years, but rely almost entirely on gross inspection of the breast tissue. Many of my colleagues report to me the same tendency. The cancer

surgeon should become highly proficient in the recognition of cancer by sight and touch. No aid from frozen sections can replace this capacity." Ewing further states that, "The pathologist should endeavor to render a clinical diagnosis and not merely a histologic report. *Hence, all available data in the case are frequently required, and the investigation will often lead to the examination of the patient and a consultation with the surgeon in charge.*"

Olch,<sup>2</sup> in his splendid chapter on "The Diagnosis of Diseases of the Breast" in Graham's "Surgical Diagnosis," writes: "The present state of early carcinoma of the breast impresses one with the fact that no surgeon should operate upon a patient with a lump in the breast unless he is able to recognize a malignant lesion. To be sure, this cannot always be done with the naked eye, in which case it is necessary to await the result of *unhurried*, thorough microscopic examination of the tissue. The writer believes that no frozen section can give any more information than can be obtained with the unaided eye, assuming, of course, that the person making such an examination is qualified to do so, and no surgeon should undertake to explore a breast tumor unless he is so qualified. . . . If the tumor is malignant the radical operation is then carried out. If there is still doubt as to the malignancy, the wound is closed and the tissue brought to the laboratory where blocks from various parts are cut and prepared for section. Frozen section is done at the request of the surgeon, but in the cases doubtful to the naked eye, it will not settle the issue. At least, one usually prefers to wait for the results of the examination of the prepared tissue. This disparagement of frozen sections cannot be blamed on faulty technic, since the writer has used the polychrome-methylene blue method for the past ten years and feels that it cannot be relied upon absolutely. The distinction must be made between the use of this method for purely diagnostic purposes, or as a means of corroborating a preoperative diagnosis. In the latter instance it is not necessary. By the method advocated here, the patient waits twenty-four hours, and if the diagnosis of carcinoma is then made, the radical operation is carried out. If the lesion proves to be benign, nothing more is done. Inasmuch as the cases about which one is in doubt



are borderline ones, and the tissue has been removed with a wide margin of normal breast, the delay of twenty-four hours does not unfavorably influence the patient's chances for cure. In no instance in which this procedure has been followed in this clinic, and in which an early carcinoma has been found, has there yet been a recurrence."

The present widespread popularity of the frozen section method is due largely to the enthusiastic writings of Bloodgood. It is particularly worthy of note that in one of the last papers<sup>3</sup> he wrote before his lamentable death he completely reversed himself and admitted that if the lesion was grossly doubtful at the time of operation there was no harm in closing the wound and waiting until experienced surgical pathologists had subjected the microscopic sections to careful study.

It is quite probable that there are occasional instances in which the frozen section serves a useful purpose but, as with many diagnostic innovations, too much has been expected of the frozen section method of rapid tissue diagnosis. The passage of time and the experience of many skillful tissue pathologists have demonstrated that the frozen section method is often capable of doing more harm than good. One aim of the American Society of Clinical Pathologists is to improve the status of cancer diagnosis in the "average" hospitals of this country. It is in such hospitals that the frozen section method is widely employed. The growing knowledge of the hazards and limitations of the frozen section diagnosis places a responsibility upon tissue pathologists to make known to their surgical brethren the fact that this diagnostic method has a very restricted field of usefulness.

—WALTER M. SIMPSON.

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## THE JOURNAL CHANGES EDITORS

The November 1936 issue of the JOURNAL marked, not only the completion of Volume 6, but the retirement of Dr. T. B. Magath as Editor-in-Chief, a position which he has held from the birth of the JOURNAL.

Dr. Magath's decision to relinquish his Editorship, made known to the Executive Committee of the Society at the Kansas City Convention, was accepted only with great reluctance and in deference to his expressed wish. In accepting the responsibility for the inauguration of the JOURNAL, Dr. Magath, in the interests of the Society, faced a task of no mean proportions. That in the short space of its existence the JOURNAL has achieved a recognized standing in its field is a tribute, not only to his ability, but to the consistent effort he has applied to its production. The Society is greatly in his debt.

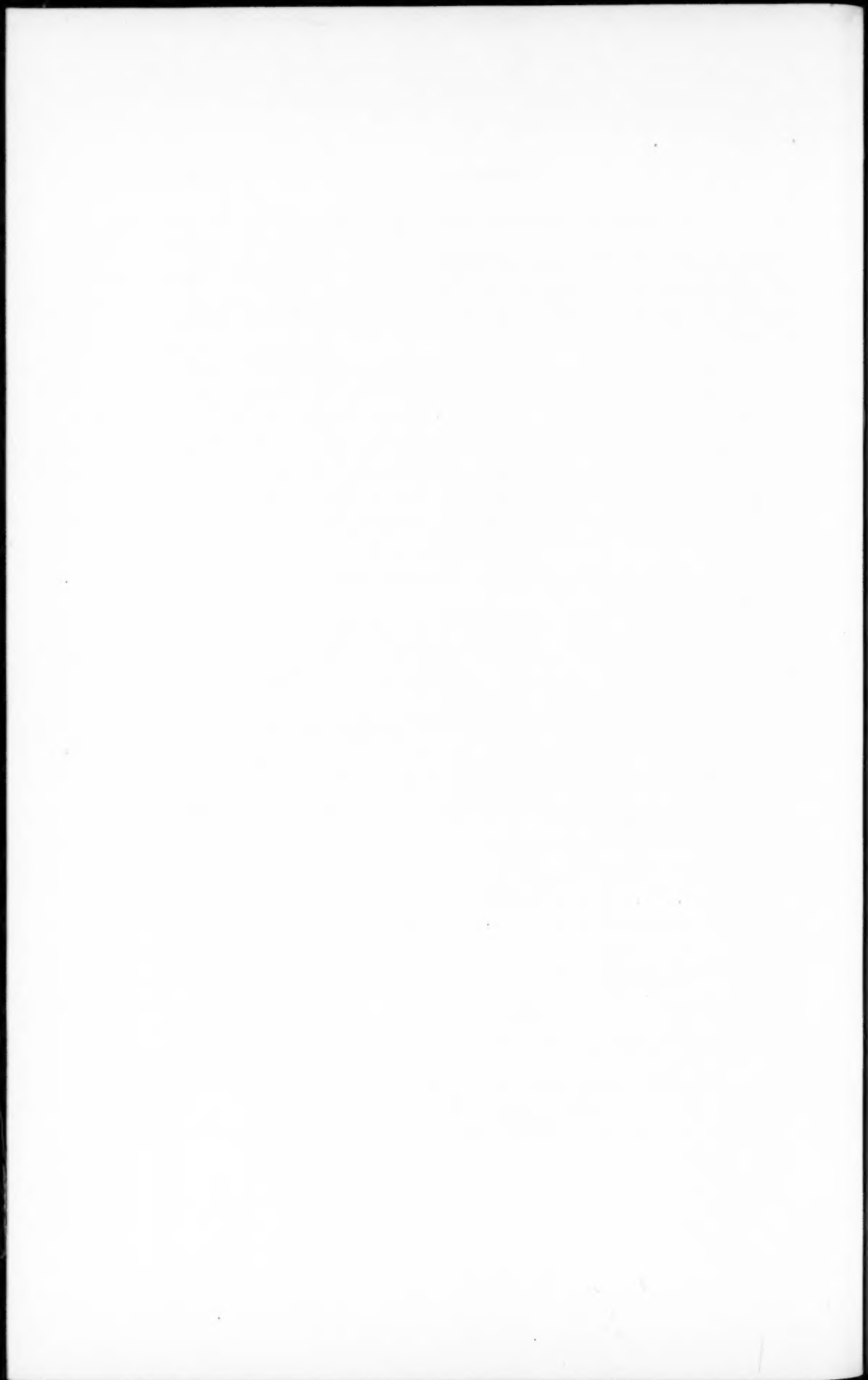
In consenting to serve as a member of the Advisory Editorial Board Dr. Magath again demonstrates his willingness to serve the Society, and the new Editor is happy in thus being able to benefit from his advice and experience.

In succeeding—but not replacing—Dr. Magath, the new editor fully appreciates the magnitude of the task before him in attempting to maintain the high standard set in the six years now passed.

To this end he bespeaks the support and coöperation of the Society at large.

—R. A. KILDUFFE.





## PHYSICOCHEMICAL FACTORS INFLUENCING THE RED CELL SEDIMENTATION RATE\*

K. YARDUMIAN

*From the Department of Pathology, Isaac Kaufmann Foundation, Montefiore  
Hospital, Pittsburgh, Pennsylvania*

Since use of the sedimentation rate of R.B.C., as a test for pregnancy by Fahreus in 1918, numerous studies and investigations on this procedure have been made both in this country and abroad. A review of part of the voluminous literature on the subject reveals that most of the papers deal with the clinical application of the test, while few discuss the factors influencing the sedimentation rate. The value in diagnosis and prognosis will not be considered in this paper; our purpose is to evaluate the physicochemical factors that may influence the rapidity of red cell sedimentation.

Studies were made on the sedimentation rate in over 2000 cases within a period of two years in both out- and in-patient departments of the hospital. The technique used was the Linzenmeier method in which 0.8 cc. of whole blood is mixed with 0.2 cc. of 5 per cent sodium citrate in a calibrated tube 5 mm. in diameter and 40 mm. in length. Sedimentation rate is the time required for the cell level to drop from 0 mark to 18 mm.

Studies were carried out on the following miscellaneous conditions and factors:

1. Observations on factors that may be classed as physical and technical in nature:
  - (a) Effects of type and quantity of anticoagulant
  - (b) Influence of temperature
  - (c) Aeration of blood
  - (d) Relation to meals

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- (e) Time element in carrying out the test
  - (f) Influence of bore and length of tube
  - (g) Position of the tube
  - (h) Centrifuging and remixing of the blood
2. Studies on chemical and cytological constituents of the blood:
- (a) Chemical constituents—non protein nitrogen, sugar, calcium, phosphorus, cholesterol, total lipids, albumin and globulin, fibrin, plasma and cell chlorides,  $\text{CO}_2$  combining power and basal metabolic rate
  - (b) Cellular—leucocytes and differential with Schilling Index, volume of R.B.C., hemoglobin, and platelet count

*Effects of type and quantity of anticoagulants.* In our studies 10 mgm. of lithium oxalate per 5 cc. of blood; 1 mgm. of heparin per 10 cc. of blood; 0.2 cc. of 5 per cent sodium citrate per 0.8 cc. of blood were used. No appreciable difference was noted between heparin and dry lithium oxalate crystals, but with 5 per cent solution of sodium citrate the rate was 5 per cent faster. This difference can be explained on the basis of dilution of the volume of plasma. The amount of these coagulants did not alter the sedimentation rate as long as there was enough to prevent coagulation without appreciably altering the volume of blood.

*Temperature.* Variation in temperature, as obtained in the incubator at  $37^\circ\text{C}$ . and the refrigerator  $4^\circ\text{C}$ ., definitely affected the sedimentation rate. If the blood was at body temperature or over, the settling of the cells was accelerated, while refrigeration had the opposite effect. The usual variation of room temperatures, between  $20^\circ$  to  $30^\circ\text{C}$ ., gave insignificant differences.

*Aeration of blood.* Venous blood was aerated for several minutes, and the sedimentation rate was entirely unaffected.

*Relation to meals.* Little difference was found in the sedimentation rate of an individual before and after eating: there was an average of 2 per cent increase in the rate after meals.

*Time element in obtaining the blood and carrying out the test.* Repeated determinations on the same sample of blood after sedimentation or centrifuging did not appreciably alter the rate when the test was performed after two hours.

*Bore and length of sedimentation tubes.* Sedimentation rate

TABLE 1  
ALBUMIN-GLOBULIN

SEDIMENTATION TIME	GRAMS PER 100 CC.			ALBUMIN-GLOBULIN RATIO
	Average	Maximum	Minimum	
0 to $\frac{1}{2}$ hour:				
Albumin . . . . .	3.85	4.70	2.20	1.6:1.0
Globulin . . . . .	2.45	4.50	1.60	
$\frac{1}{2}$ to 1 hour:				
Albumin . . . . .	3.81	5.10	2.29	1.8:1.0
Globulin . . . . .	2.12	3.80	1.20	
Over 1 hour:				
Albumin . . . . .	4.01	5.50	3.17	1.4:1.0
Globulin . . . . .	2.75	3.50	1.30	

TABLE 2  
CHOLESTEROL

SEDIMENTATION TIME	AVERAGE MILLIGRAMS PER 100 CC.
<i>hours</i>	
0 to $\frac{1}{2}$	179.5
$\frac{1}{2}$ to 1	162.0
Over 1	207.0

TABLE 3  
PLASMA CO<sub>2</sub>

SEDIMENTATION TIME	AVERAGE PERCENTAGE
<i>hours</i>	
0 to $\frac{1}{2}$	45.4
$\frac{1}{2}$ to 1	53.0
Over 1	52.0

TABLE 4A  
CELL VOLUME

SEDIMENTATION TIME	PERCENTAGE OF CELLS	MINIMUM	MAXIMUM
<i>hours</i>			
0 to $\frac{1}{2}$	31.5	14.0	47.1
$\frac{1}{2}$ to 1	37.5	19.0	52.8
Over 1	40.1	21.0	61.5

was the same in tubes having diameters varying from 5 to 11 mm. on identical columns of blood. Fluctuation was noticed in wide or too narrow tubes.

TABLE 4B  
VOLUME OF PACKED CELLS IN RELATION TO SEDIMENTATION RATE

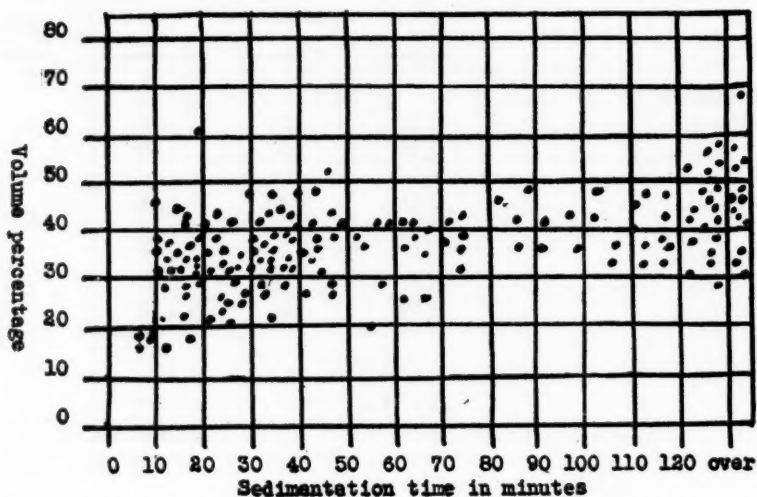
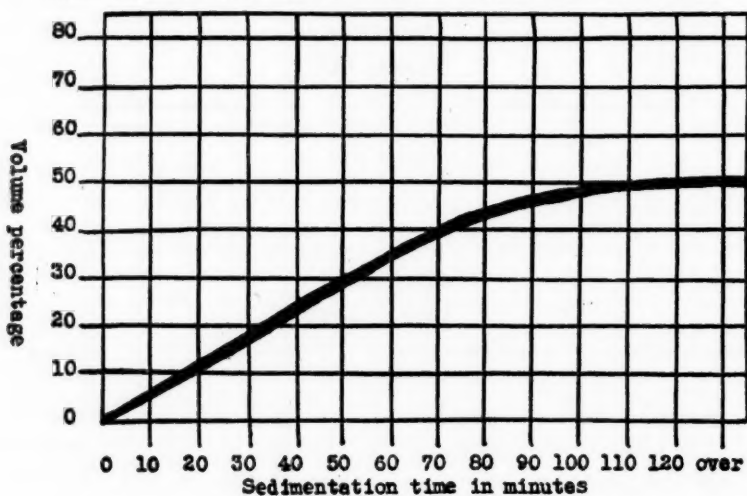


TABLE 4C  
CELL VOLUME CURVE FOR CORRECTION OF SEDIMENTATION RATE



*Position of the tube.* This has a very definite effect on the rate of sedimentation of R.B.C. Any deviation from the perpendicular position accelerates the rate, the variation at times amounting to 50 per cent.

In a series of fifty cases no correlation could be found between the sedimentation rates and the CO<sub>2</sub> combining power, the basal metabolic rate, the non-protein nitrogen, or the calcium or phosphorus content. The blood sugar level had very little effect on the sedimentation rate, there being a moderate tendency to

TABLE 5  
PLASMA AND CELL CHLORIDES

SEDIMENTATION TIME	AVERAGE MILLIGRAMS PER 100 CC. IN TERMS OF SODIUM CHLORIDE	
	Cell	Plasma
<i>hours</i>		
0 to $\frac{1}{2}$	307	575
$\frac{1}{2}$ to 1	306	569
Over 1	313	581

TABLE 6  
LEUCOCYTE COUNT

SEDIMENTATION TIME	AVERAGE	MAXIMUM	MINIMUM
<i>hours</i>			
0 to $\frac{1}{2}$	14,080	19,000	4,780
$\frac{1}{2}$ to 1	13,366	37,000	6,600
Over 1	9,840	67,000	5,600

TABLE 7A  
PLASMA FIBRIN  
Normal 300 to 375 MGM.

SEDIMENTATION TIME	MILLIGRAMS PER 100 CC.		
	Average	Maximum	Minimum
<i>minutes</i>			
0 to 30	502	730	310
30 to 60	410	520	290
60 to 90	387	750	270
Over 90	359	350	180

sluggish sedimentation when the blood sugar values were over 200 mgm. per 100 cc.

*Albumin-globulin.* 75 cases. Determined by the colorimetric method of Wu and Ling as modified by Greenberg.<sup>1</sup> (See table 1.)

*Cholesterol.* 53 cases. Determined by colorimetric total cholesterol in Bloor's modified technique.<sup>2</sup> (See table 2.)

*Plasma CO<sub>2</sub> combining power.* 40 cases. Van Slyke technique. (See table 3.)

*Cell volume.* 143 cases. Method used: 5 to 10 cc. of citrate blood centrifuged  $\frac{1}{2}$  hour at 2000 revolutions per minute. Table

TABLE 7B  
PLASMA FIBRINOGEN. 163 CASES  
Normal 350 to 450 mgm.

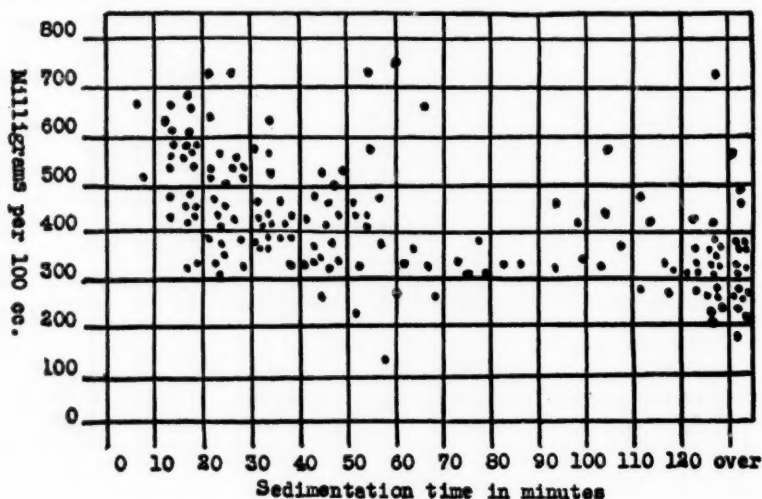


TABLE 8A  
LIPIDS OF BLOOD PLASMA  
Normal 450 to 550 mgm.

SEDIMENTATION TIME	MILLIGRAMS PER 100 CC.		
	Average	Maximum	Minimum
minutes			
0 to 30	746	1028	417
30 to 60	625	1111	334
60 to 90	604	639	536
Over 90	526	958	311

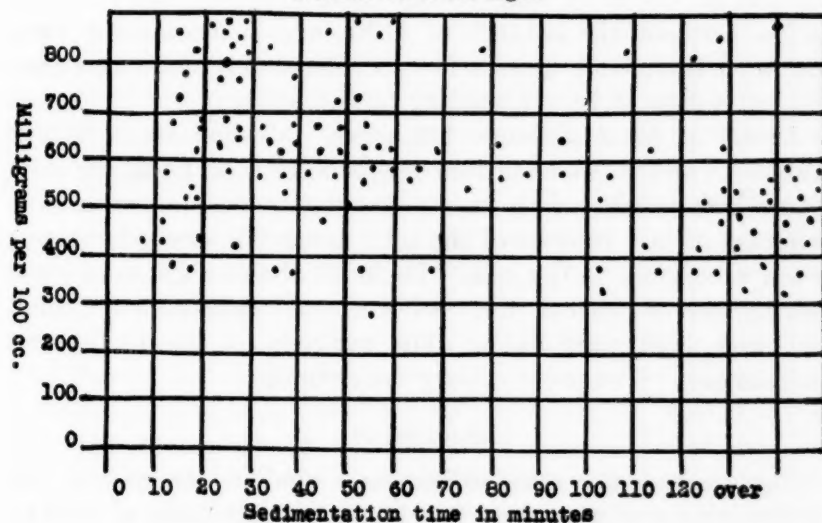
4A shows that the concentration of cells has a marked effect on the sedimentation rate: the smaller the volume of the packed cells, the faster the sedimentation rate. (See tables 4A, 4B and 4C.) Table 4C is intended to make corrections on the sedimentation rate according to the cell volume.



TABLE 8B  
COMPARATIVE FIGURES OF PLASMA FIBRIN AND TOTAL LIPIDS

SUBJECT NUMBER	SEDIMENTATION TIME minutes	MILLIGRAMS PER 100 CC.	
		Fibrin	Lipids
1	26	350	1000
2	64	360	555
3	19	330	686
4	59	130	905
5	43	350	677
6	33	380	644
7	30	380	666
8	22	340	861
9	17	320	639
10	19	410	708
11	44	330	775
12	17	480	728

TABLE 8C  
TOTAL PLASMA LIPIDS. 116 CASES  
Normal 450 to 550 mgm.



*Plasma and cell chlorides.* 280 cases. Method used: Whitehorn.<sup>3</sup> Table 5 shows definitely that cell and plasma chlorides are fairly constant in health and disease, and apparently do not influence the sedimentation rate.

*Leucocyte count.* 250 cases. (See table 6.)

*Leucocytes.* The total count has no definite effect on the rate of sedimentation. Cases included leukemia and leukopenia. There is no parallelism between the leucocyte count and the sedimentation rate, except that a moderate leukocytosis and a rapid sedimentation rate may be caused by the same condition—a suppurative process. A normal count is more common with a normal sedimentation rate. Similarly the Schilling Index and the differential count have no bearing on the rate of sedimentation, except that the same conditions which cause rapid sedimentation may also cause a shift to the left.

*Platelets.* The total count has no effect, except that clumping of the platelets on smears is more frequently seen when sedimentation rate is rapid than when normal. The counts ranged between 100,000 and 500,000.

*Plasma fibrin.* 163 cases. Method used: Precipitation of fibrin, colorimetric method of Cullen and Van Slyke.<sup>4</sup> Tables 7A and 7B definitely suggest that there is a very important relation between the content of fibrin and sedimentation rate, although there were quite a few individual determinations that did not follow strictly according to the scale.

*Lipids of blood plasma.* 116 cases. Method used: Bloor.<sup>5</sup> Tables 8A and 8C indicate that the average total lipids are relatively high in cases with fast sedimentation and low with slow sedimentation. In spite of the total average figures, there were a few exceptions to the rule. Table 8B shows a group of cases having low or normal fibrin with a rapid sedimentation rate, but with high total lipids. The majority of the cases were pregnancies. Two were miliary tuberculosis.

#### DISCUSSION

There are many standardized and modified techniques for determining and recording the sedimentation rates of R.B.C. In spite of variations in the length and width of tubes and in the type and concentration of anticoagulants, variations in the rate of sedimentation are negligible. Observations on a limited number of specimens where parallel tests were performed on the

same sample of blood with the three most commonly used techniques, Linzenmeier, Cutler, and Westergreen, indicate that the results check very closely within the limits of technical errors. These findings corroborate those of Greisheimer and his co-workers.<sup>6</sup>

Observations on the influence of temperature, aeration of blood, meals, time element in carrying out the test, and position of the tube coincided with the findings of Wintrobe.<sup>4</sup>

Theories on surface tension, capillary attraction and differences in electrical charges of diverse elements of the blood influencing the rate of sedimentation of R.B.C. have been suggested by various authors—Fahreus, Hoeber, Mond and others. They claim that red cells normally carry a negative charge and thus repel each other. These authors state that in certain alterations of the blood electrolytes and colloids, this negative electric charge is diminished and thus the decreased repulsion of the cells facilitates sedimentation. Furthermore, these colloids have a greater absorbing power for the alkaline salts in the plasma and thus also is the negative electric charge of the R.B.C. diminished, effecting a more rapid sedimentation. In this work the commonly known colloids and crystalloids of the blood were studied in relation to the rate of sedimentation. This will be fully discussed in the latter part of this paper.

The so-called auto-agglutination of R.B.C. is, in no sense, a true serological agglutination. The clumps are merely aggregations of R.B.C. or a rouleaux formation which leaves the individual cell intact, so easily separated, in various media. In true agglutination the cells are never separable if once clumped. The theory that antibodies have some influence on the rate of sedimentation as suggested by Lendertz has very little support. But this phenomenon of rouleaux formation or clumping is one of the most interesting factors that enters into the rate of sedimentation of R.B.C. Physical law of surface area and surface tension of particles in suspension is readily applicable in sedimentation rate. As long as the individual R.B.C. are separate and held in suspension, the rate will be very much delayed; the sooner and larger the clump or rouleaux formation, the faster

the sedimentation rate is. The total sum of surface area of individual R.B.C.s is three to four times larger than when they are aggregated.

*Cytological studies.* Variation in leucocyte count has no influence on sedimentation time, although the average count may be slightly higher in rapid sedimentation because of the presence of leucocytosis in the majority of infections. Schilling Index and sedimentation rate have a similar noncausal relationship R. J. Griffin.<sup>8</sup> The weight and hemoglobin content of the cells showed no correlation with the rate of sedimentation in this series of cases. Similar findings are recorded by Greisheimer<sup>9</sup> and others. The number of R.B.C. has a definite influence on the rate of sedimentation. This has been established either by determining the volume of packed cells or by counting the number of R.B.C. The lower the cell volume, the faster the rate of sedimentation. (See chart for corrections.) Similar studies and corrections have been recommended by Walter,<sup>10</sup> Boerner,<sup>11</sup> and Wintrobe.<sup>12</sup>

Variation in the number of platelets is not a constant factor, although they are increased in pneumonia and inflammations of serous cavities where sedimentation time, in general, is fast.

Apprehension and emotional upsets have been found to be factors in the rate of sedimentation in children.<sup>13</sup> We did not have the opportunity to make such observations in our cases.

Chemical constituents, such as calcium, phosphorus, non protein nitrogen, CO<sub>2</sub> combining power, cell and plasma chlorides, and cholesterol had apparently no influence on the rate of sedimentation. A blood sugar of over 200, however, had a moderate effect in retarding the sedimentation rate.

Further experimental work by suspending the cells in 8 per cent egg albumin, normal salt solution, and isotonic solutions of KCl and CaCl<sub>2</sub> resulted in a retardation or prevention of sedimentation, while in emulsified oily solutions the sedimentation time was accelerated.

Partial replacement of the plasma with patient's serum or partial replacement with normal salt solution also caused marked retardation of the sedimentation time.

Fahreus' experimental studies established the fact that the formation of large aggregations of red cells is responsible for fast sedimentations and this phenomenon is almost exclusively dependent on the nature of the plasma. This opinion is well substantiated by later experimental work of Hoeber and Mond, and of Linzenmeier. Fahreus found that erythrocytes settle faster in plasma than in serum. Further studies led him to the conclusions that albumin has only a slight enhancing influence on sedimentation whereas globulin and fibrinogen have a greater effect on sedimentation time.

Bruchsaler in his studies on relative sedimentation rate and blood fibrin in infants and pregnant women, found that maternal blood showed 250 to 500 mgm. of fibrin while the blood of the new born infant contained 64 to 125 mgm. per 100 cc. of plasma. In pregnant women, sedimentation rate was fast, while in infants it was slow. S. L. Ellenberg<sup>14</sup> states that normal newborn infants 7 to 24 hours old have longer sedimentation times than older infants, blood fibrin probably being the most important factor.

Starlinger<sup>18</sup> and Frisch<sup>19</sup> found further evidence of the importance of fibrinogen content of plasma in sedimentation rate by simple experiment. When some of the fibrinogen is absorbed by kaolin or charcoal the sedimentation rate decreases. These latter experiments cannot be accepted as proof that the fibrinogen is the important factor, for the substances mentioned absorb other constituents than fibrinogen, and bring about decided changes in the electric potentials of the plasma.

In our experimental work glass beads were substituted for kaolin in order to defibrinate the blood. Our results showed definite slowing of sedimentation rate after defibrination.

The majority of authors on the subject agree that blood fibrinogen content is one of the most important factors influencing the rate of sedimentation. Among those, are Dochez,<sup>20</sup> Krosing,<sup>21</sup> Bruchslaer and Hunt.<sup>15, 16</sup> On the other hand Pinner and co-workers<sup>17</sup> expressed the belief that there is no definite relationship between blood fibrin and the sedimentation time of R.B.C.

The normal value of plasma fibrin, according to the literature, varies between 100 to 350 mgm. per 100 cc. depending on the method used by Sussman.<sup>22</sup>

Our average for normals ranged higher than these figures, the extreme being 250 to 400 mgm. and the average 300 mgm. per 100 cc. of plasma. In the group of studies on fibrinogen content with sedimentation rate (163 cases), the majority of rapidly sedimenting specimens had high fibrin contents, but no precise parallelism existed. Over 20 per cent of the cases were exceptions to the rule; these were cases of slow sedimentation rates with high fibrin contents and vice versa. Parallel fibrin and total lipoids of plasma on a group of cases were run. It was found, that those cases with rapid sedimentation rate that had low fibrin, generally had, high total lipoids, especially in late pregnancies where no appreciable increase of fibrin was seen, but relatively high lipid values were found. On the other hand, cases with pneumonia or inflammations of the serous cavities such as peritonitis, pleuritis, pericarditis, meningitis and synovitis, invariably showed high fibrin.

Kurten states that the addition of cholesterol to blood increases the sedimentation rate and that lecithin has the opposite effect. Lasch emphasized the accelerating action of cholesterol on sedimentation rate. The former showed that this action was independent of alterations in the proteins. He produced alimentary hypercholesteremia in rabbits and found that they had high sedimentation rates. Lasch thought that high sedimentation rates were found in all diseases in which the blood cholesterol value was high. On the other hand Pinner and co-workers<sup>17</sup> in their studies did not confirm Lasch's findings. They found corresponding results in only 10 out of 39 cases studied.

Our studies are in agreement with Pinner and co-workers and contrary to Grossman and Lasch. No correlation between the amount of cholesterin and sedimentation rate was found (see table 2). This was repeatedly proved among diabetics and pregnancies, where the cholesterin content is generally increased. Total lipoids of plasma has more direct effect on sedimentation rate than the cholesterin content alone. This condition was especially noted in cases where, in spite of fast sedimentations, the fibrin contents were low while total lipoids were high (see table 8B).



In our series, although there is a definite relationship between the fibrin and lipid content of the blood and its sedimentation rate, increased amounts of either accelerating the rate, this correlation was not quantitative or proportional.

#### SUMMARY

Studies have been made on clinical conditions in over 2000 cases evaluating the influence of various factors on the sedimentation rate of R.B.C.

The results were classified arbitrarily into three groups:

1. Factors that did not appreciably influence the rate of sedimentation:
  - (a) The use of different anticoagulants
  - (b) The use of the various standardized techniques for determining the rate
  - (c) Variations in temperature between 20° to 30°C. (room temperature)
  - (d) Remixing and resedimentation
  - (e) Variation in the chemical constituents: blood sugar, non protein nitrogen, calcium, phosphorus, CO<sub>2</sub> combining power, cholesterol, plasma and cell chlorides, and albumin-globulin ratio
  - (f) Variation in the number of white blood cells and platelets
2. Factors that moderately affect the rate:
  - (a) Delay of over two hours in running the test slowed the rate
  - (b) Variation in the bore and length of the sedimentation tube
  - (c) Extreme temperature variation below 20° and above 30°C. cold slowing and heat hastening the settling of cells
  - (d) Albumin-globulin content of blood—high values increasing the rate
  - (e) Marked hyperglycemia of 200 mgm. or more slowing the rate
3. Factors that markedly affect the rate:
  - (a) High fibrin generally accelerated the rate of sedimentation
  - (b) High total lipoids in certain cases had the same effect as fibrin
  - (c) Volume of packed cells—the smaller the volume, the quicker the sedimentation rate
  - (d) Deviation of the sedimentation tube from perpendicular position

The test, although simple in technique, depends on so many complex and variable factors that there is no parallelism between the physical and chemical characteristics of the blood and the sedimentation rate. Cell volume, fibrin content and total



lipoids markedly influence the rate of sedimentation. Even these factors however, have no constant effect, there being many exceptions that could not be explained by quantitative analysis.

These factors probably alter the surface tension, capillary attraction or electrical charges of the cell or plasma causing rouleaux formation or clumps and thus the rate of sedimentation in both physiological and pathological conditions.

It is, therefore, inconceivable that a phenomenon so readily influenced by many variable factors, should be of appreciable diagnostic and prognostic value.

*Note:* I wish to express my appreciation for the kind assistance of Dr. J. Finegold, Dr. J. W. Mendoza, and Dr. J. R. Sugerman, and also the technical assistance of Nancy C. Newman, B.S., and to the internes of the hospital who coöperated fully during their laboratory service.

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## BRONCHIOGENIC CARCINOMA\*

OSBORNE ALLEN BRINES AND JOHN CARL KENNING

*Departments of Pathology and Radiology, Receiving Hospital, Detroit, Michigan*

In the past decade primary malignancy of the lung, or bronchus, has risen from comparative obscurity to a position of importance. The lesion is now frequently encountered and possesses interesting clinical and pathological features. Its frequent insidious onset, late development of symptoms, short clinical course, and its frequent simulation of acute and chronic pulmonary infection make its recognition difficult unless clinicians are conscious of its true prevalence and are sufficiently alert in the differential diagnosis of pulmonary lesions.

The incidence of primary pulmonary malignancy seems to be higher in Central Europe than elsewhere. In Germany lung cancer constitutes approximately 10 per cent of all malignancy, isolated reports having shown the incidence to be as high as twenty per cent. In England and Northern Europe the incidence is considerably lower. This geographic distribution is of interest because at Receiving Hospital nearly 50 per cent of patients with primary intrathoracic malignancy have been of Central European birth or extraction, which is much higher than the normal distribution of these nationalities in the hospital population. In the past five years at Receiving Hospital, in the routine examination of twelve thousand surgical specimens, the bronchus occupied fifth place as a primary site of malignancy, the specimens consisting of bronchoscopic biopsies. In three thousand consecutive autopsies at this hospital during the same period of time, the lung (bronchus) ranked second only to the stomach as a primary site of cancer. During the past three years since the organization of the Tumor Clinic at this hospital,

\* Read at the Fifteenth Annual Meeting of the American Society of Clinical Pathologists, at Kansas City, May 8-10, 1936.

seventy-three cases of primary lung cancer have been registered, an incidence of 7.8 per cent of all proven malignancy.

The etiology of this disease has been carefully considered by various authors.<sup>1</sup> The influenza epidemic of 1918-19, exhaust gases from automobiles, tar on roads, tobacco smoking and chronic tuberculosis have been offered as contributing factors toward the increasing incidence of lung cancer. It seems fair to say, however, that none of these can be accepted as definitely causative. The usual principles of carcinogenesis probably operate here and are represented chiefly by chronic pulmonary inflammation, mainly bacterial but conceivably chemical in origin. Occupation does not seem to affect the incidence. The

TABLE I  
CHIEF PRIMARY LOCATIONS OF CARCINOMA IN 3000 CONSECUTIVE AUTOPSIES

	NUMBER OF CASES
Stomach.....	56
Lung.....	25
Intestine.....	23
Liver.....	17
Pancreas.....	16
Prostate.....	15

Low incidence of breast, rectal and uterine cancer is due to the fact that cases are transferred to another hospital for X-ray and radium therapy and prolonged nursing care.

majority of our patients have been laborers, but it must be considered that the material has been accumulated in a charity hospital.

#### PATHOGENESIS

Although our understanding of pulmonary malignancy is by no means complete, we are apparently much more accurately informed than we were a few years ago. It is not difficult to realize how easy it was at one time to consider the majority of these lesions as sarcomas, and it was a considerable advance to adopt a classification which included adeno-, alveolar, squamous and "oat cell" carcinoma. Such a classification was based on a

fairly satisfactory belief that adenocarcinoma developed from columnar epithelium of the bronchus or from mucous glands, alveolar carcinoma from alveolar epithelium, "oat cell" carcinoma from a specific basal cell in the bronchial wall and that the origin of squamous cell epithelioma depended upon metaplasia from columnar to squamous epithelium.



FIG. I. CARCINOMA OF BRONCHUS WITH INVOLVEMENT OF TRACHEOBRONCHIAL NODES

It has become quite generally agreed, especially in America, that primary cancer of the lung is bronchiogenic. The belief was expressed by Ribbert<sup>2</sup> in 1905 and by Weller<sup>3</sup> in 1929 that the different cell types of bronchiogenic carcinoma can be explained by differentiation of a common parent cell rather than by histogenesis. The presence of glandular, papillary, or mucin-secreting

forms in a pulmonary neoplasm does not necessarily mean derivation from a glandular structure. That squamous epithelium is frequently found lining chronically infected bronchi and bronchiectatic cavities cannot be denied and yet the site of squamous cell carcinoma does not usually coincide with the site at which the greatest amount of metaplasia generally occurs. Squamous epithelium lining a bronchus, the wall of which is invaded by "oat cell" carcinoma, is not an uncommon finding. Metaplasia is a term frequently over-used and is a process which is probably not so simple as we have been inclined to believe. The fact that more than one type of epithelium may be found in walls of normal or inflamed bronchi has led to an attempt to explain the existence

TABLE II

NEW ADMISSIONS TO TUMOR CLINIC AT RECEIVING HOSPITAL FROM OCTOBER, 1932 TO MAY, 1936 (SIX MOST COMMON PRIMARY SITES)

	NUMBER OF CASES
Total number of cases.....	936
Stomach.....	122
Cervix.....	122
Breast.....	74
<i>Lung</i> .....	73
Skin.....	65
Rectum.....	62

of more than one type of bronchiogenic carcinoma on the theory that each type is derived directly from a preexisting similar cell which was either normal or abnormal for that particular site. The possibility of successfully demonstrating a primary lung cancer of alveolar origin intrigues the pathologist, and yet the existence of alveolar epithelium is not a universally accepted fact.

Attempts have been made to classify bronchiogenic carcinoma into two main groups: "oat cell" and squamous cell. This is undeniably simple and no doubt possesses certain clinical advantages. The term "oat cell" is being used here somewhat apologetically. Although it is a widely accepted term, it is definitely objectionable and does not represent a clear-cut entity in tumor diagnosis. It merely refers to a group of poorly differentiated



FIG. II. PHOTOMICROGRAPHS SHOWING ADENOCARCINOMA, SQUAMOUS CELL CARCINOMA AND "OAT-CELL" CARCINOMA  
FOUND IN A SINGLE MICROSCOPIC SECTION THE SOURCE OF WHICH CAN BE SEEN IN FIGURE I



bronchiogenic carcinomas and, if properly interpreted, serves as a convenient but roughly descriptive term. Unfortunately, however, all bronchiogenic carcinomas are not oat cell or squamous cell, inasmuch as some are found which are composed of columnar cells and are characterized by a definite acinar or papillary pattern. Squamous cell carcinoma is a fairly clear-cut entity here as elsewhere, but the so-called "oat cell" type is far from being a homologous lesion.

Weller,<sup>3</sup> in 1929, presented a classification of primary carcinoma of the lung based entirely on differentiation and not on histogenesis. He represented the course of differentiation of a common parent cell by the letter "Y." This undifferentiated cell

TABLE III  
RACE AND SEX  
(68 cases)

	NUMBER	PER CENT
<i>Color:</i>		
White.....	63	92.7
Colored.....	5	7.3
<i>Sex:</i>		
Male.....	63	92.7
Female.....	5	7.3

in his schema occupies the lower extremity of the stem and the slightly or poorly differentiated neoplasms, formerly called sarcomas, are represented by the stem of the "Y." The crotch of the "Y" represents the point at which some evidence of differentiation into columnar or squamous cells has been reached. The left arm of the "Y" represents differentiation into columnar epithelium and the extreme upper portion of the left arm represents ent-differentiation into papillary and mucin-secreting types. Along the right arm of the "Y" various stages of differentiation into squamous cells is supposed to occur, progressing to ent-differentiation into cornifying epithelioma at the top. Weller at that time classified fourteen carcinomas of the lung according to

this schema, and in the succeeding five years found no lesion that could not be so classified.

One's first reaction to such an explanation is liable to be unfavorable but a careful study of cases encountered convinces one of its adaptability. The difficulty with this, as well as any other attempt at classification of pulmonary malignancy is the marked variation of cell morphology in the same tumor. Minor varia-

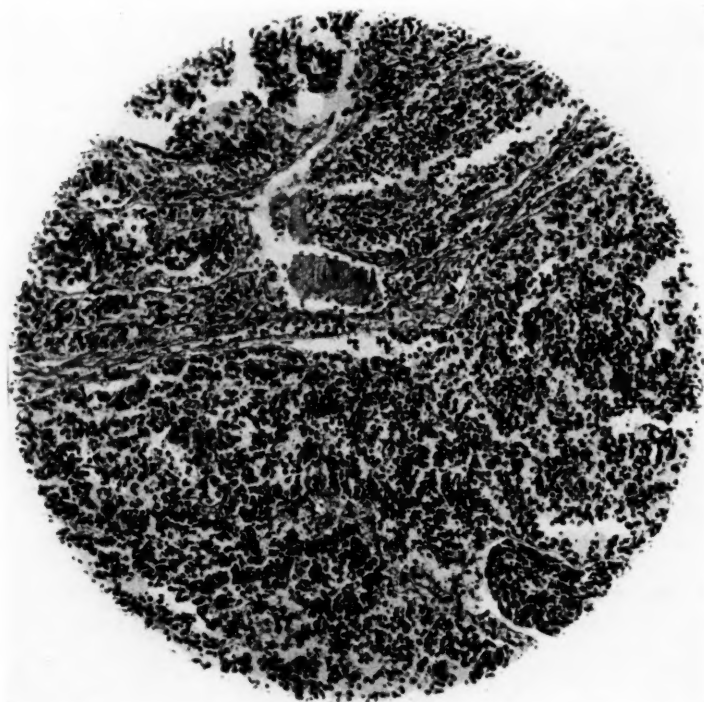


FIG. III. PHOTOMICROGRAPH OF UNDIFFERENTIATED BRONCHIOGENIC CARCINOMA

tions in the stage of differentiation of these tumors is frequently seen and this is comparable to conditions found in malignant tumors elsewhere. An exhaustive microscopic study of each lesion encountered would emphasize the fact that these variations are occasionally quite conspicuous. It is not impossible to find squamous, "oat cell" and adenocarcinoma in the same microscopic section, and the finding of two distinct cell types is

comparatively common. The variations of cell types in the same tumor are unquestionably due to multiple stages of differentiation and constitute the highest type of proof that the various microscopic patterns in primary carcinoma of the lung are entirely the

BRONCHIOGENIC CARCINOMA

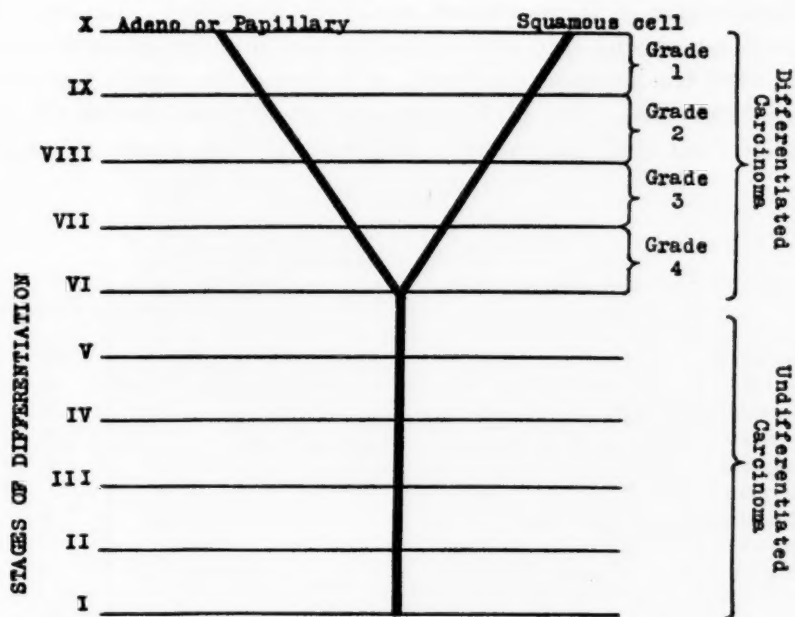


CHART I. The different cell types of bronchiogenic carcinoma are accounted for by the differentiation of an immature or embryonal parent cell.

At the left are represented ten stages in differentiation which are indicated in Roman numerals.

The letter Y is used to illustrate. The stem represents five stages of undifferentiated carcinoma which includes the so-called "oat-cell" variety.

The arms of the Y represent differentiation into adeno- or squamous cell carcinoma. At the right are given the corresponding conventional grade of malignancy of squamous cell carcinoma.

result of differentiation of a common ancestral cell. That differentiation should be so commonly into squamous cells is not remarkable inasmuch as there is reason to believe that the columnar epithelium of the bronchus does not represent full potentiality of differentiation, and that further differentiation

into squamous epithelium should be a reasonable biological expectation.

#### PATHOLOGY

It seems that for practical purposes, in dealing with rapidity of growth, liability to metastasis and radiosensitivity, our present classification of these tumors could be simplified. Division of these neoplasms into differentiated and undifferentiated types conveys the necessary amount of information which possesses practical value. The undifferentiated group would include the so-called "oat cell" carcinoma and grade III and grade IV squa-

TABLE IV  
NATIONALITY  
(60 cases)

	NUMBER	PER CENT
American.....	22	36.7
Polish.....	17	28.3
Russian.....	5	8.3
Austrian.....	4	6.7
Jugoslav.....	4	6.7
German	2	3.3
Hungarian		
Scotch		
English		
Each.....		
	60	99.9

mous cell carcinoma. Undifferentiated carcinoma of the lung usually begins at the hilum and tends to infiltrate the wall of the bronchus, invading the peribronchial tissue without producing ulceration of the mucosa. For this reason the term "infiltrating type" is often employed. The lumen may be narrowed from hypertrophy of the wall but it is rarely completely occluded. The primary tumor does not generally grow to a large size but metastasizes early to the tracheobronchial nodes and frequently to the liver, upper abdominal lymph nodes, adrenals, bone and kidneys. Radiographically the lesion is usually found near the hilum and extends in centrip-

etal fashion to the lung parenchyma by way of the peribronchial lymphatics. The mediastinal structures are usually not displaced. Late in the disease there is frequently involvement of the pleura with pleural effusion and hemothorax. In this type of lesion the use of lipiodol is a frequent aid to the roentgenologist.



FIG. IV. PHOTOGRAPH OF TYPICAL SPECIMEN OF UNDIFFERENTIATED BRONCHIOGENIC CARCINOMA

The differentiated type, including both adeno- and squamous cell carcinoma, may begin at some distance from the hilum, frequently in the middle of the upper lobe or at the apex. The primary tumor is often bulky and necrosis is common, frequently producing cavitation giving the gross appearance of an abscess, in the wall of which neoplastic tissue is often accidentally found upon microscopic examination at necropsy. Ulceration of the

bronchial mucosa usually occurs and proliferation of neoplastic tissue into the lumen with occlusion is a frequent occurrence. Because of the intrabronchial accumulation of living neoplastic cells, aspiration metastasis is possible and involvement of the opposite lung occasionally occurs. Metastasis is slower and frequently absent. The tracheobronchial nodes are usually not conspicuously involved. Radiographically, the most common picture seen in this obstructive type is that of atelectasis. The degree of atelectasis depends upon the degree of bronchial occlusion. The classical pictures of complete obstruction are: a homogeneous opacity of the affected side; retraction of the mediastinal contents to the affected sides, associated with retraction of the ribs, narrowing of the intercostal spaces, elevation

TABLE V  
AGE DISTRIBUTION

AGE	NUMBER	PER CENT
<i>years</i>		
30 or less	1	1.4
30-40	10	14.8
40-50	18	26.4
50-60	27	39.8
60-70	10	14.8
70-80	2	2.8

and restriction of the diaphragm on the side involved, together with compensatory emphysema on the opposite side. Cavitation occurs frequently in the larger tumors of this type.

#### SUMMARY

Bronchiogenic carcinoma deserves recognition as one of the commoner diseases. Neoplastic lesions of the bronchus are invariably malignant. Of 44 cases classified morphologically, 21 were squamous cell, 6 adeno- and 17 undifferentiated carcinoma. In addition two tumors contained areas representing all three types, two both adeno- and squamous cell and one squamous cell and undifferentiated carcinoma. In this group of cases the right lung was involved primarily in approximately 60 per cent.

The incidence of bronchiogenic carcinoma in any general hospital will closely parallel the aggressiveness of the radiographic and bronchoscopic services and the extent to which these departments understand the prevalence of this lesion and look for its presence. Fifty per cent of the cases were bronchoscoped and in 73 per cent of these a diagnosis of bronchiogenic carcinoma was made.

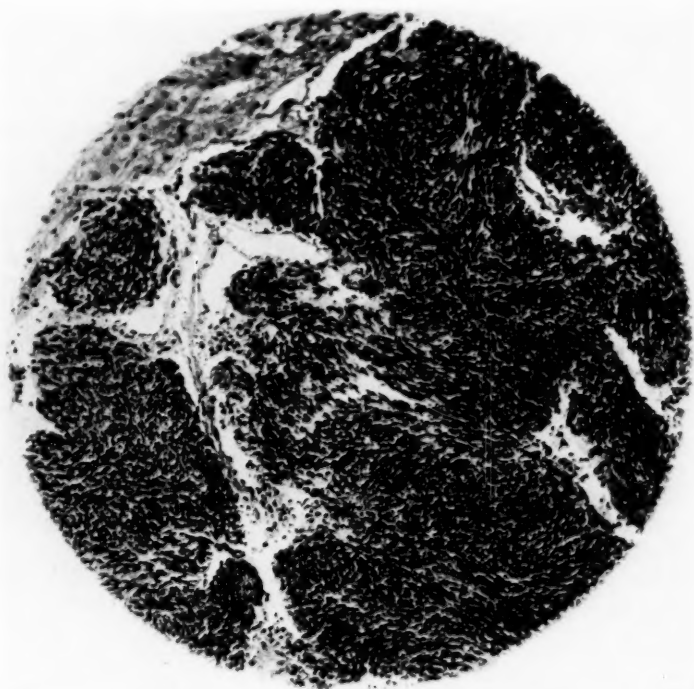


FIG. V. POORLY DIFFERENTIATED BRONCHIOGENIC CARCINOMA COMPOSED OF SPINDLE SHAPED CELLS

Same case as figure IV

It is essential for the clinician to suspect the possibility of bronchiogenic carcinoma in patients over thirty years of age with pulmonary abscess, in patients who fail to recover from lobar pneumonia, and in patients with persistent cough, chest pain, hemoptysis and steady weight loss. Twenty-five and eight-tenths per cent of the patients in this series had initiating symp-



toms of an acute respiratory infection or pneumonia. History of weight loss of from a few pounds up to sixty pounds was obtained in 79.4 per cent. Cough was complained of in 87 per cent, being productive in about half and non-productive in the other half. While only 18.9 per cent complained of frank hemoptysis, 48.3 per cent stated that they had coughed up varying amounts of blood at some period of their illness. Sixty and two-tenths per cent complained of chest pain of which there were two out-

TABLE VI  
CHIEF COMPLAINTS

	NUMBER	PER CENT
Total number of patients.....	73	
Cough.....	36	50.3
Chest pain.....	31	44.8
Weight loss.....	24	31.0
Hemoptysis (frank).....	17	18.9
Dyspnea.....	10	14.7

TABLE VII  
DURATION OF SYMPTOMS

AGE	NUMBER	PER CENT
1 month or less	3	4.2
1-3 months	19	27.8
3-6 months	27	39.7
6-9 months	7	9.2
1 year or over	5	7.0

Shortest history was 2 weeks; longest was 3 years.

standing types: dull aching type and sharp pleuritic type. Weakness was generally marked, 67.6 per cent registering this symptom.

The commonest physical findings were lag on the affected side, dullness to flatness on percussion, diminished or absent breath sounds, voice sounds and fremitus. These findings were almost always associated with atelectasis or fluid or both.

Results of treatment have been discouraging. Only an occasional lesion is operable. The undifferentiated carcinomas

should be radiosensitive, a quality which is offset by the fact that they are characterized by early and widespread metastasis. Two patients in this series are alive at the end of three and one-half years, one being completely asymptomatic and the other comparatively so. Both received deep X-ray therapy. The first was an undifferentiated carcinoma and the other squamous cell, grade I. Inasmuch as this lesion would be ordinarily considered radioresistant, the clinical result was probably due to the low grade malignancy and the healing of the associated pneumonitis.

Recognition is hereby given Dr. William A. Hudson who performed the bronchoscopic examinations in these cases.

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## EVALUATION OF RESULTS OF FLOCCULATION TESTS FOR SYPHILIS IN THE RECENT AMERICAN CONFERENCE

### OUTLINE OF STUDIES FOR STANDARDIZATION OF THESE TESTS\*

B. S. KLINE

*From the Laboratories of the Mount Sinai Hospital, Cleveland, Ohio*

The Committee evaluating the results of serodiagnostic tests for syphilis in the recent American Conference has presented three reports<sup>1,2,3</sup> and has concluded that the study "indicates relatively equal value to the clinician of efficient complement fixation tests and efficient flocculation tests as applied to either blood or spinal fluid specimens." Another evaluation outlined below, based upon criteria no less applicable, indicates that two flocculation tests (slide tests and Kahn tests) gave somewhat more specific and definitely more sensitive results with blood specimens and equally specific and decidedly more sensitive results with spinal fluid specimens than did the better complement fixation tests. Still another evaluation of the results was published recently by Moore<sup>4</sup> and is discussed below.

Since the degree of specificity of the various tests for syphilis is the most important thing that is evaluated, the nature of the control cases used and the evaluation of doubtful results in non-syphilitic bloods are of the utmost importance.

The Evaluation Committee, in its various reports,<sup>1,2,3</sup> has estimated the specificity of the blood tests from one group only, namely the normal presumably nonsyphilitic individuals, 152 in number, and have not counted doubtful reactions in non-syphilitic serums against the specificity of a test. On this

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basis, nine blood tests had a specificity of over 99 per cent with five giving absolutely specific results as follows: Specificity 100 per cent: Brem (comp. fix.), Kahn (floc.), Kline (floc.), Kolmer (comp. fix.), Williams (comp. fix.). Specificity 99.3 per cent: Hinton (floc.), Rein (floc.), Ruediger (comp. fix.), Weiss (floc.). The others in order of specificity are Lufkin and Rytz (floc.) 98.7 per cent, Eagle (floc.) 98 per cent, Johns (floc.) and Kurtz (floc.), each 96.7 per cent.

In contrast to an evaluation of tests based upon a small control group of normal individuals, the 435 control cases in the League of Nations Conference in Copenhagen<sup>5</sup> included no normals whatever but was composed of presumably nonsyphilitic individuals with other venereal disease, skin disease, pregnancy, mental disorder, tuberculosis, tumor and fever (listed in order of number with the largest group first). Again in the 304 control cases in the League of Nations Conference at Montevideo the controls included, in order of number presumably, nonsyphilitic patients with mental disorder, skin disease, normals, other venereal disease, pregnancy and fever. It can be maintained, therefore, that there is good precedent for considering Table A, Appendix III of the Detailed Report of Results<sup>3</sup> of the American Conference, to furnish the best basis for the evaluation of specificity of the various tests, inasmuch as it includes not only 152 normal presumably nonsyphilitic individuals but also the presumably nonsyphilitic individuals with tuberculosis, malignant neoplastic disease, fever, jaundice, pregnancy, during menstruation and during the intermenstrual interval (468 in all).

Moore<sup>4</sup> has based his evaluation of the specificity of the various tests upon these 468 control cases and states, "The increased number of nonsyphilitic serums provides a much more accurate basis of evaluation of specificity." For the same reason the evaluation of specificity offered in his paper is also based upon the larger control group.

Moore<sup>4</sup> has disposed of the unsatisfactory results of all blood tests in leprosy (40 to 76 per cent) and in active malaria (8.6 to 20.6 per cent) in the following way, "Aside from syphilis, the only disease conditions which give positive results with serologic

tests for syphilis are yaws, relapsing fever, leprosy (all frequently), and malaria (infrequently)." (The unsatisfactory results of all the tests in the twenty-seven leprosy cases in the Montevideo Conference were likewise excluded from the control cases by the Montevideo Conference.)<sup>6</sup>

Another factor of importance in evaluating the results of the American Conference is the interpretation of the  $\pm$  reactions. Concerning these the Committee reported, "The Committee has found the evaluation of doubtful reports impracticable. A logical method is lacking for determining the amount of credit to be assigned or the deduction to be made in respect to such reports," and "Throughout this study specimens giving doubtful reactions are included in the columns headed 'specimens examined' but are not counted as positive or partially positive reports in determining percentages of positive reports or percentages of negative reports. Although in this study the doubtful reports have been given a negative rating, the Committee recognizes that in a clinical practice a doubtful report may often be of value."<sup>3</sup>

Quite similarly the Committee of the Montevideo Conference evaluated the  $\pm$  reactions as follows,<sup>6</sup> "Although the sensitiveness and specificity of the serodiagnostic method are determined primarily by the percentage of positive reactions obtained in cases of syphilis and in control cases, reactions which were neither positive nor negative ( $\pm$ ) also play an important part. On the one hand, a great number of  $\pm$  reactions signify that, in these cases, the question put by the clinician to the serologist remains unanswered; on the other hand, a  $\pm$  reaction in a case of treated syphilis, for example, should be considered as a weakly positive reaction. It is important to know, therefore, the proportion of the cases in which the  $\pm$  reaction denotes syphilis—that is to say, to determine the relative diagnostic value of  $\pm$  reactions, since this value is not apparent from the absolute number of  $\pm$  reactions obtained in cases of syphilis and in control cases. It is determined by calculating the ratio between the percentage of  $\pm$  reactions in cases of syphilis and in control cases."

From the evaluation of these two committees, it would appear

that the  $\pm$  reactions in nonsyphilitic cases are to be considered as doubtful reactions and not to be counted against the specificity of a test, and that  $\pm$  reactions in syphilitic cases are to be considered as weakly positive reactions having a definite but indeterminable value.

Moore's<sup>4</sup> evaluation of  $\pm$  reactions is as follows: "Though it is obvious that a doubtful result in a known syphilitic patient is not as convincing as a positive result, it is nevertheless scorable as a partial success for the competing serologist. Conversely, while a false doubtful result in a nonsyphilitic individual is not as disturbing to the clinician as a false positive test, it is nevertheless disturbing enough and as such scorable as a partial failure for the serologic test in question." This evaluation by Moore is at variance with that indicated in his graph which lists the tests in the order of their specificity. Here false doubtful results are apparently counted as much against a test as false positive reactions.

In his evaluation Moore concludes: "If one utilizes as a satisfactory standard of specificity that chosen by the serologists (both of the American and the Montevideo Conferences), i.e., less than 1 per cent false positive tests in nonsyphilitic persons, seven tests qualify as satisfactory. If, however, false doubtful results are added to the false positives, only four are satisfactory: Kline diagnostic, Kolmer complement fixation, Kahn diagnostic and Brem complement fixation."

Moore further concludes: "For the increased sensitivity demanded for the special purposes enumerated in the text, the Ruediger complement fixation test and the Hinton and Kline exclusion flocculation test (Rein) are the methods of choice." (The special purposes referred to are (1) testing blood donors, (2) use in special syphilis clinics having expert clinical interpretation available, and (3) in following cases of syphilis under treatment.)

In the evaluation of the results of the American Conference outlined below, the specificity of the various tests is determined by the results in the larger group of control cases as given in Appendix III of the Detailed Report of Results<sup>3</sup> of the Conference



and as employed by Moore<sup>4</sup> in his evaluation. It is furthermore based on the following consideration of the  $\pm$  reactions:

1. In nonsyphilitic serum this reaction is considered a doubtful result and although scored as a partial failure it is counted much less against a test than a false positive reaction and any number of false doubtful reactions is counted less against a test than one false positive reaction.
2. In known syphilitic serum this reaction is considered a weakly positive reaction. Since a  $\pm$  reaction is of some significance in any stage of known syphilis and because a weakly positive reaction is to be expected early in the disease and also for some time before the completion of adequate treatment, a  $\pm$  reaction in syphilitic serum although of indeterminable worth is given a value of 75 per cent as against 100 per cent for a positive or strongly positive reaction.

Graph A, Appendix III, of the Detailed Report of Results of the American Conference, based on Table A, shows the blood tests arranged in alphabetical order. Based upon the criteria given above, figures 1 and 2 present the tests in the order of their specificity and sensitivity and as in the Montevideo Conference report<sup>6</sup> the tests are divided into (1) those with less than 1 per cent false positive reactions and (2) those with more than 1 per cent false positive reactions.

In this evaluation, seven of the thirteen tests are considered to have shown a satisfactory specificity (99 per cent or better) (Kline, Kahn, Weiss, Williams, Kolmer, Brem, Rein) but none to have shown a 100 per cent specificity. This evaluation differs decidedly from that of the Evaluation Committee based upon 152 normal controls in which five tests had 100 per cent specificity.

In fairness to the diagnostic slide test and Kahn standard test, however, it should be said that the 0.2 per cent false positivity recorded against them was due to a positive result in a case of tuberculosis in which ten of the tests were positive, one doubtful and two negative. The two tests giving negative results were the least sensitive of those evaluated. If this case were con-



# ERRATUM

Vol. 7, No. 2, March, 1937

Pages 139, 140, 142, 143, 148, 149: Because of printer's error the legends to figures 1-6 were omitted.

## BLOOD TESTS. EVALUATION IN ORDER OF SPECIFICITY\*

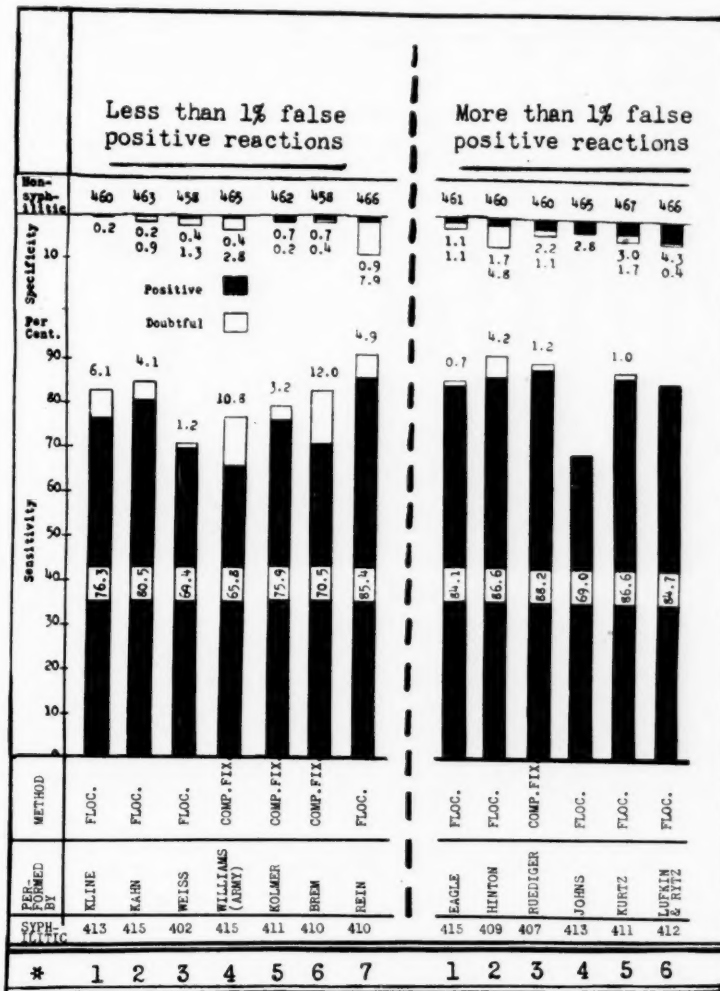


FIG. 1. Weak reactions ( $\pm$  or  $+$  in the old nomenclature, and  $\pm$  in the new nomenclature) considered as doubtful reactions in non-syphilitic cases and as weakly positive reactions in syphilitic cases.

# BLOOD TESTS. EVALUATION IN ORDER OF SENSITIVITY\*

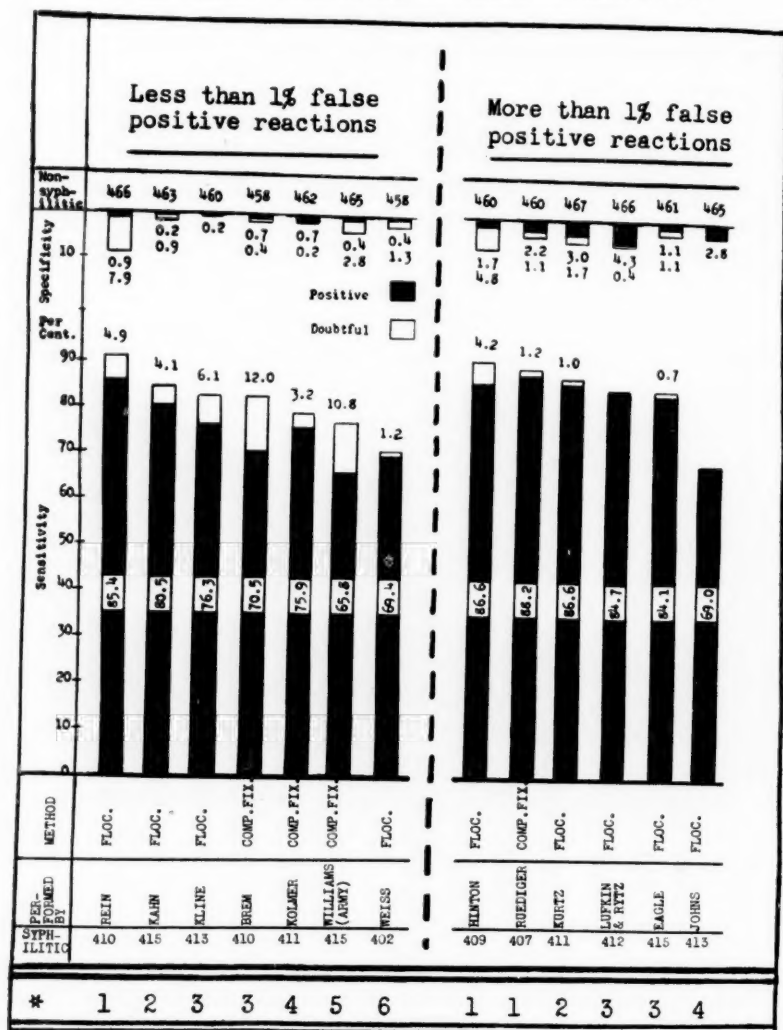


FIG. 2. Weak reactions ( $\pm$  or  $+$  in the old nomenclature, and  $\pm$  in the new nomenclature) considered as doubtful reactions in non-syphilitic cases and as weakly positive reactions in syphilitic cases.

# SPINAL FLUID TESTS. EVALUATION IN ORDER OF SPECIFICITY\*

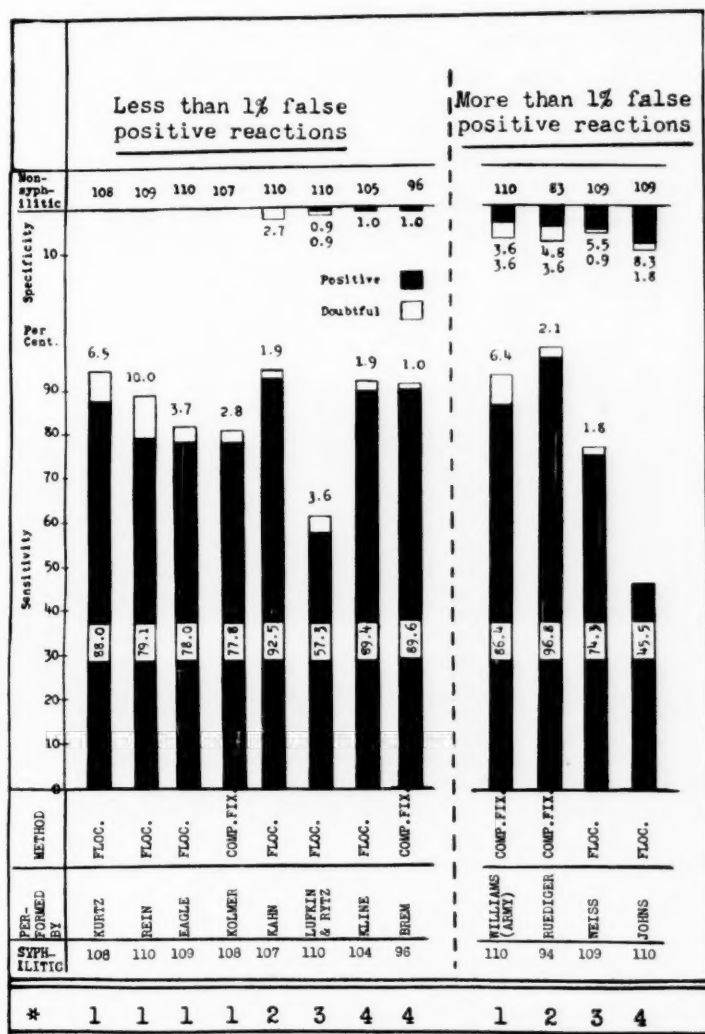


FIG. 3. Weak reactions ( $\pm$  or  $+$  in the old nomenclature, and  $\pm$  in the new nomenclature) considered as doubtful reactions in non-syphilitic cases and as weakly positive reactions in syphilitic cases.

# SPINAL FLUID TESTS. EVALUATION IN ORDER OF SENSITIVITY\*

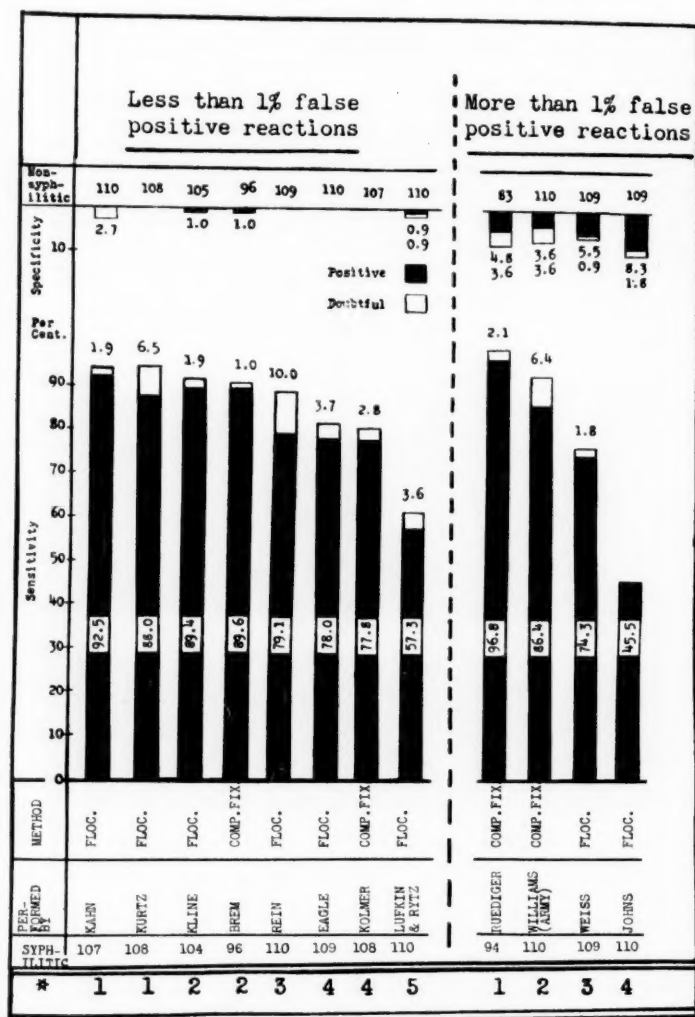


FIG. 4. Weak reactions ( $\pm$  or  $+$  in the old nomenclature, and  $\pm$  in the new nomenclature) considered as doubtful reactions in non-syphilitic cases and as weakly positive reactions in syphilitic cases.

ANTIGEN BASES (10 PER CENT) EMULSIFIED IN A MINIMAL QUANTITY  
OF WATER

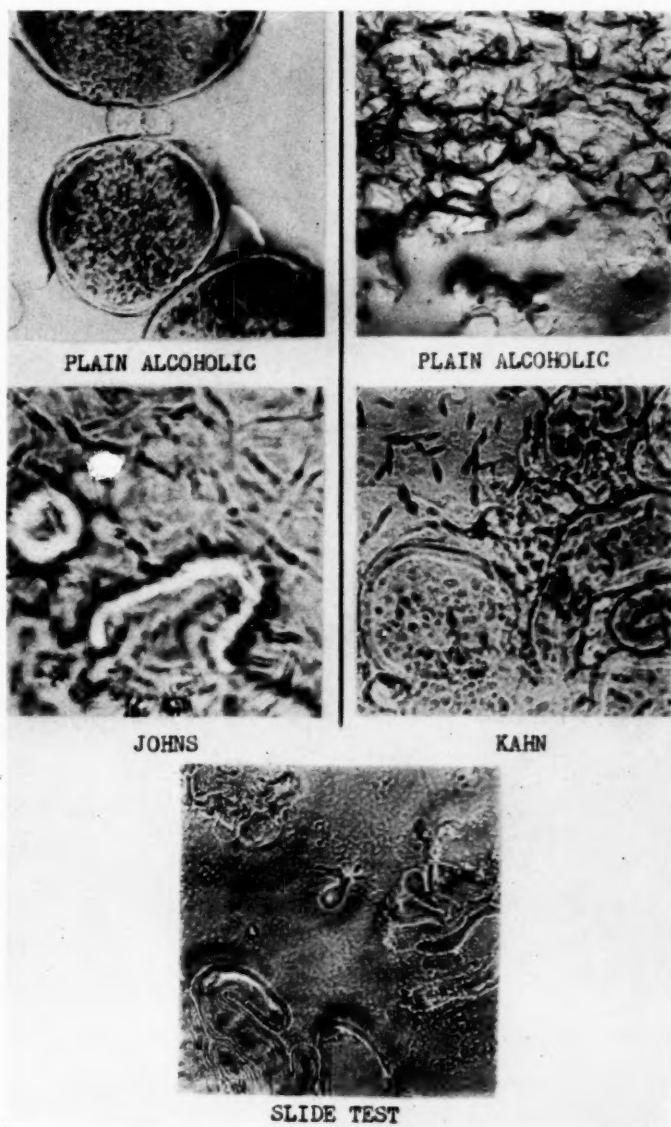
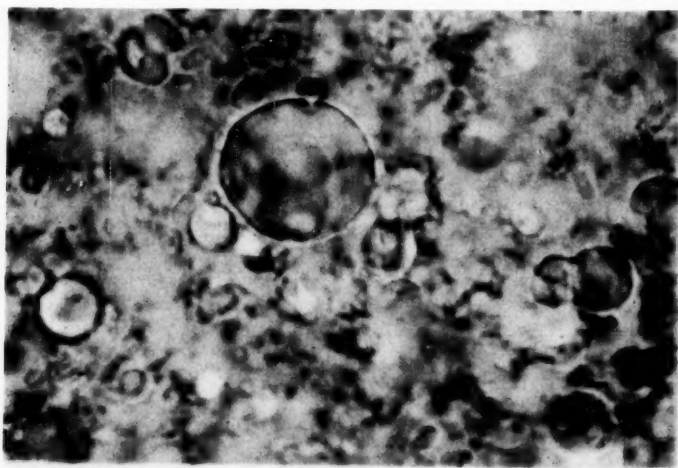
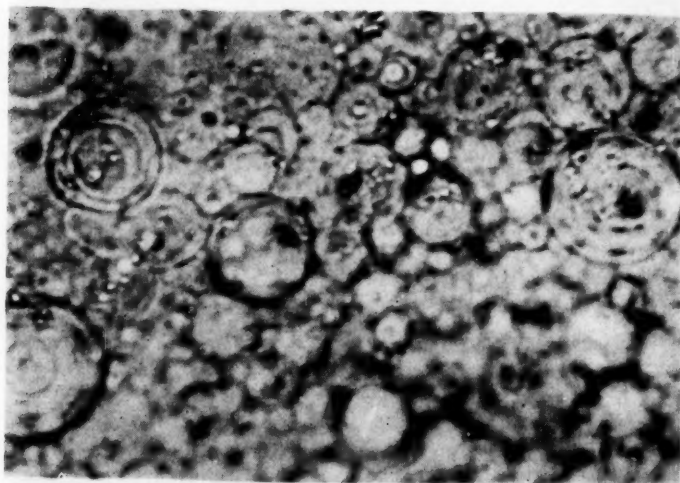


FIG. 5

SLIDE TEST ANTIGENS EMULSIFIED IN A SMALL QUANTITY OF WATER



STANDARD



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FIG. 6

sidered as not certainly nonsyphilitic and not included in the series, the diagnostic slide test would be the only test of those evaluated with 100 per cent specificity. Of the seven tests

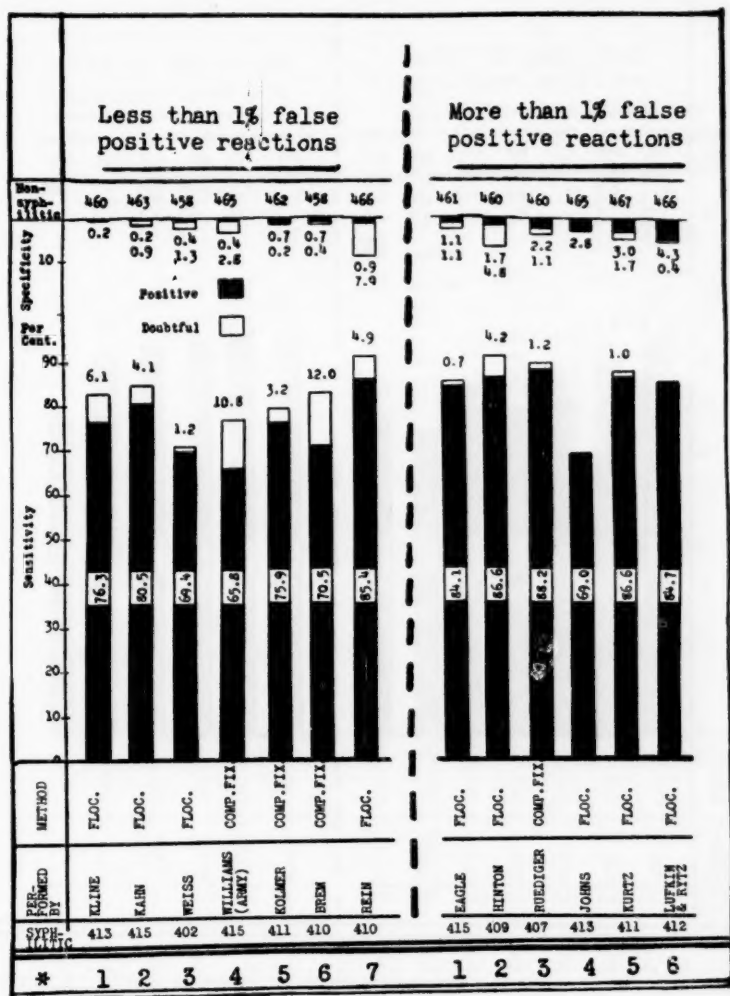


FIG. 1

with satisfactory specificity, the three giving the most sensitive results were flocculation tests performed by Rein, Kahn and Kline.



The evaluation study has made it clear that no one blood test possesses a maximum specificity and a maximum sensitivity,

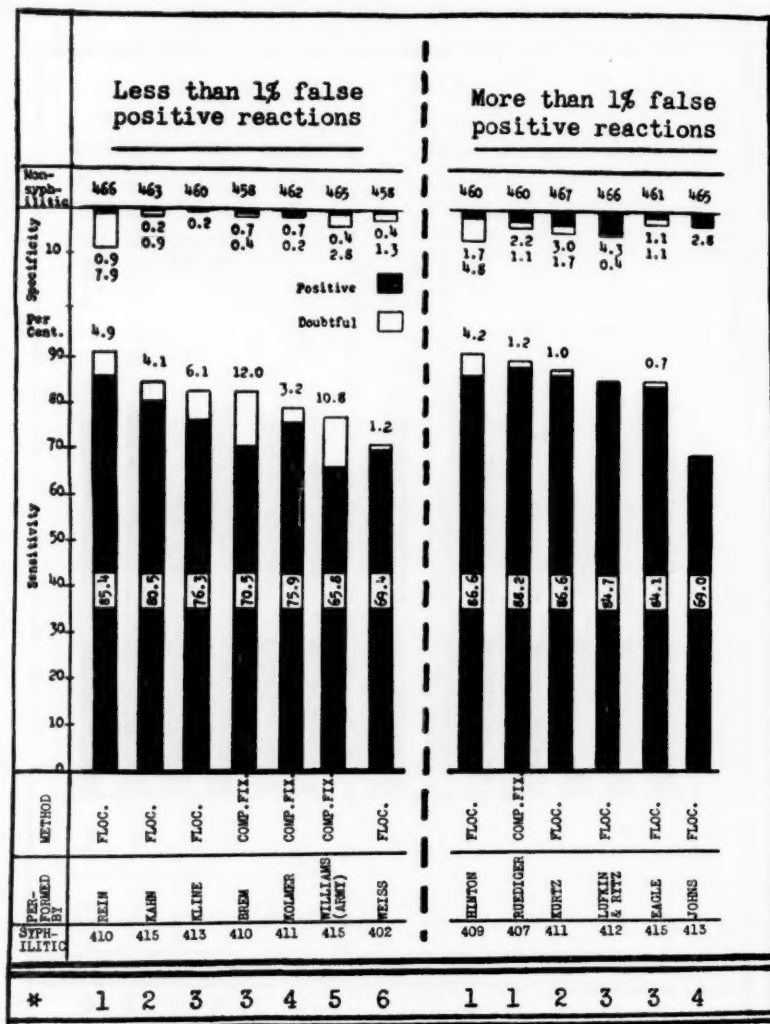


FIG. 2

and that as concluded by the Evaluation Committee:<sup>1,2,3</sup> "There is some evidence that a properly performed highly sensitive flocculation test might be used routinely for the purpose of

excluding the likelihood of syphilis. If a negative result is obtained by such a method, it is quite likely that it will be negative by any other method. If the test yields a positive result it should be repeated and compared with one or more highly specific flocculation or complement fixation tests."

The exclusion and diagnostic slide tests of blood serum conform very well to these requirements. The Kahn presumptive test is less valuable as an exclusion test than is the Kahn standard test as a diagnostic test.

Although in the group with more than 1 per cent false positive reactions, the Eagle test results indicate the value of this test as an exclusion test. The Lufkin and Rytz test is also sensitive but gave 4.3 per cent false positive results. The Hinton test with desirable sensitivity and 1.7 per cent false positivity is less valuable as an exclusion test because of the time required for its performance.

Concerning the spinal fluid examinations, the evaluation presented below does not differ significantly from that of the Evaluation Committee but is based upon the same criteria as for the blood tests and upon Table A, Appendix III, and Graph B, of the Detailed Report of Results<sup>3</sup> instead of upon Table 4, of the first report of results<sup>1</sup> employed by the Evaluation Committee. Whereas in Graph B, mentioned above, the spinal fluid tests are arranged in alphabetical order, in figures 3 and 4 they are arranged in the order of their specificity and of their sensitivity.

In this evaluation, eight of the twelve spinal fluid tests showed a satisfactory specificity (99 per cent or more with four giving absolutely specific results as follows: Specificity 100 per cent: Eagle (floc.), Kolmer (comp. fix.), Kurtz (floc.), Rein (floc.) and then in order: Kahn (floc.), Lufkin and Rytz (floc.), Kline (floc.), Brem (comp. fix.). Of the eight spinal fluid tests with satisfactory specificity, the three giving the most sensitive results were flocculation tests performed by Kurtz, Kahn and Kline.

Based upon the evaluation given above, two flocculation tests (the slide tests and the Kahn tests) gave somewhat more specific and definitely more sensitive results with blood specimens than

did the better complement fixation tests and these flocculation tests gave equally specific and decidedly more sensitive results

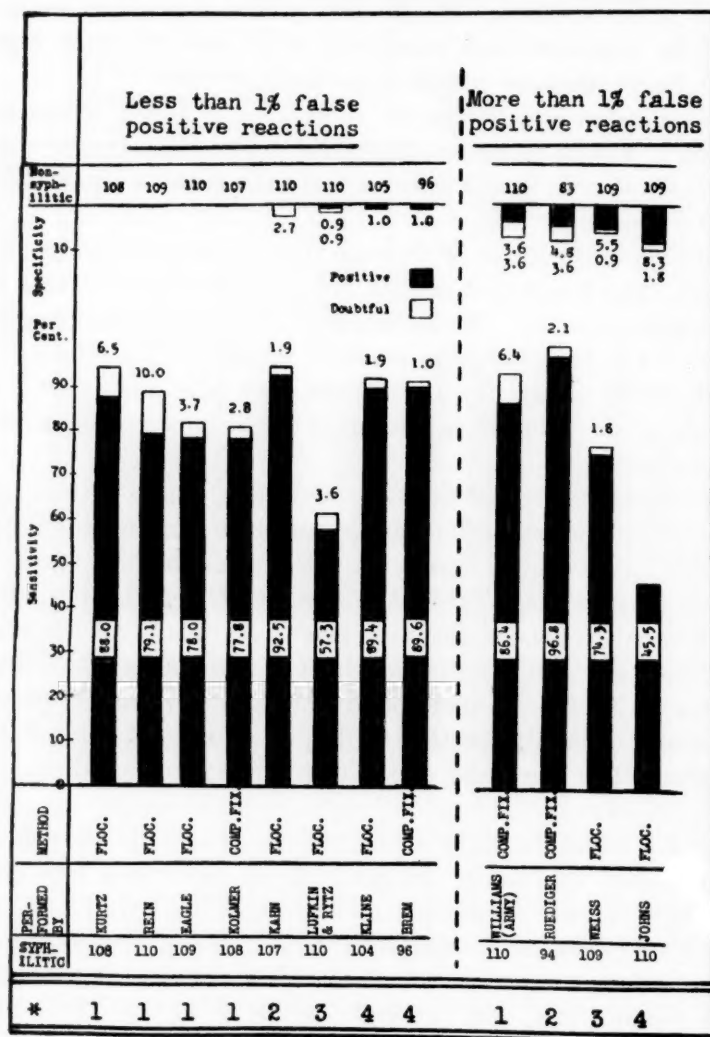


FIG. 3

with spinal fluid specimens than did the better complement fixation tests.

In further evaluating the results of the American Conference,

one conclusion of the Evaluation Committee<sup>1,2,3</sup> of interest particularly to serologists is the following: "As a secondary con-

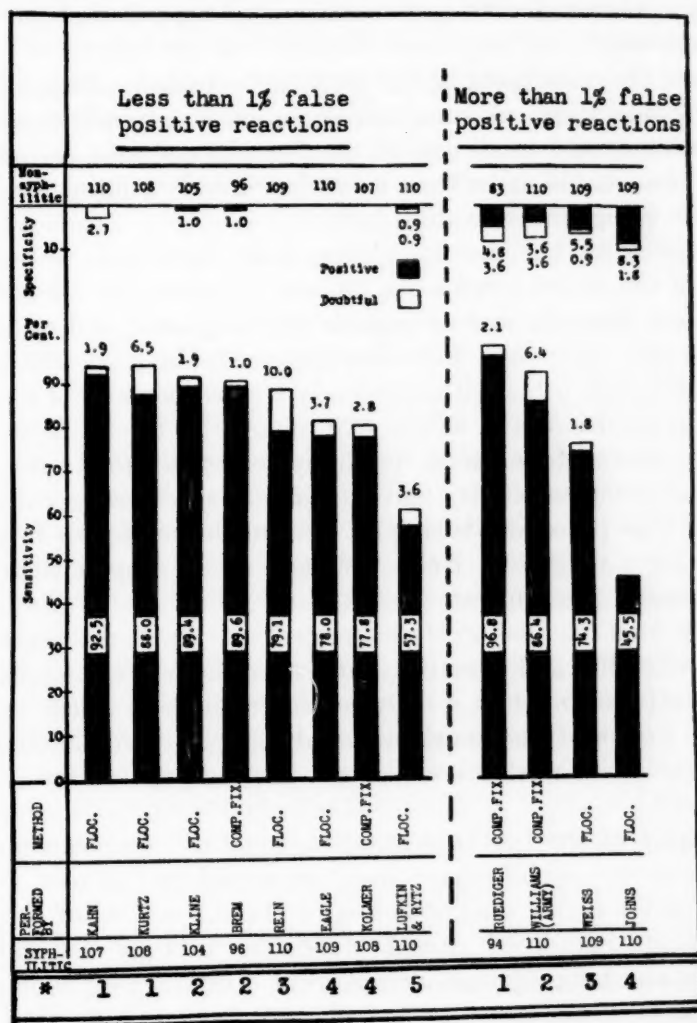


FIG. 4

sideration in the choice of one or more tests for general use, due regard should be given to the cost, rapidity, and ease of performance. No consideration was given to these factors in this

evaluation project. Certain tests which may be performed rapidly on blood specimens appeared to yield results comparable to those obtained with tests requiring a longer period for their performance."

From the standpoint of the serologist especially, such secondary considerations as comparative simplicity, rapidity, ease of performance and small cost of the flocculation tests, are advantages that should make them more desirable for routine use than are the complement fixation tests.

Incidentally, the two flocculation tests that gave the best results of the recent Conference possess the secondary advantages in fullest measure, and as regards the diagnostic and exclusion slide tests, these are done simultaneously in small chambers adjoining each other with differently treated portions of a single antigen emulsion, and within five minutes of the mixture with serum, results of maximum specificity and satisfactory sensitivity and maximum sensitivity and satisfactory specificity are obtainable. This procedure is simpler than mixing materials in three tubes for a diagnostic Kahn test, and much simpler than any complement fixation test.

Now that the better flocculation tests have demonstrated their reliability and sensitivity, and since the results may be had in a matter of minutes, a daily routine with them is less tedious to the serologist and much more advantageous to the clinician than the twice weekly complement fixation test routine widely practiced.

In spite of the fact that many satisfactory complement fixation tests for syphilis have been developed in the past thirty years and a better understanding of the interaction of the five ingredients concerned gained, difficulties in fully determining and establishing optimal complement fixation test conditions still exist. In contrast to complement fixation tests with five ingredients, some requiring careful titration and one requiring tedious preparation, the flocculation tests require but two ingredients and the interaction of these is already fairly well established so that their standardization may be possible.

## OUTLINE OF STUDIES FOR STANDARDIZATION OF FLOCCULATION TESTS FOR SYPHILIS

Although at first thought it may seem a comparatively simple matter to determine the optimal conditions for a flocculation test for syphilis requiring as it does antigen emulsion and patient's serum only, the fact is that sufficient variables are at play in the preparation and the use of the antigen emulsion to make its standardization a difficult matter. Studies to achieve this end would include:

- I. The preparation of an antigen free of adventitious substances.
- II. The preparation of optimal antigen emulsions.
- III. The determination of optimal test conditions.

*I. Antigen free of adventitious substances*

Perhaps the first step advisable in attempting to standardize flocculation tests for syphilis would be to make careful physical, chemical and biological studies of the antigens now in use and of others also with the objective of securing the most potent fraction in heart powder extract, free of adventitious substances to use as a base in the preparation of antigen emulsions. At this time the base could be used in the various flocculation tests in about the same concentration as in the present antigens employed (8.75 per cent slide test, 1.65 per cent Kahn test, 0.85 per cent Johns test).

A few antigen studies of the type that may be helpful in achieving the goal of a standard antigen base are as follows:

**I. PLAIN ALCOHOLIC EXTRACT OF HEART POWDER**

When the alcohol of plain alcoholic extract of heart powder is evaporated off the residue is found oily, vaseline-like, but sticky and dark brown. When rubbed and shaken in about a hundred parts of water, there is very little emulsification. The sticky wax in ten parts of absolute ethyl alcohol emulsifies in water in the form of highly refractile globules (visible through the microscope). The more water, the smaller the globules. In a minimum quantity of water (five to ten small drops of extract, one small drop of distilled water [free of appreciable sediment after evaporation]), the globules are highly



refractile light gold, and as the free fluid evaporates, the globules become larger and an abundant feathery precipitate develops within and about the globules (see fig. 5). Emulsified in a small quantity of water, the globules are smaller than those in the minimal quantity of water, are highly refractile, light gold. Some appear colorless.

The acetone soluble portion of these extracts containing neutral fats, fatty acids, cholesterin, etc., ordinarily weighs at least three times the acetone insoluble fraction.

Plain alcoholic extract antigens especially when cholesterinized frequently give false positive results in tests for syphilis.

## 2. ALCOHOLIC EXTRACTS OF HEART POWDER PREVIOUSLY EXTRACTED WITH ETHER OR ACETONE

Antigens obtained by first extracting heart powder with ether or acetone and subsequently with alcohol as for the Kahn and Sigma tests, although far superior to plain alcoholic extract, contain variable quantities of ether insoluble, or acetone or alcohol soluble adventitious substances.<sup>7,8</sup>

When the alcohol of Kahn antigen base is evaporated off, the residue is found soft, yellow brown, waxy. When rubbed or shaken in about one hundred parts of water, complete emulsification occurs readily. The wax in ten parts of absolute ethyl alcohol when emulsified in a minimum quantity of water (five to ten small drops of extract, one small drop of distilled water) shows light gold elongated globules much less refractile than those of plain alcoholic extract. As the free fluid evaporates there is a precipitation out of it, of numerous small needle-like crystals (see fig. 5).

The soft yellow brown antigen wax obtained by evaporation of Kahn alcoholic heart extract, is separable into two fractions by extraction with boiling ether. The soluble portion yields a dark brown wax having the same physical, chemical and biological properties as the dark brown acetone insoluble wax described below (standard slide test antigen wax). The ether insoluble adventitious fraction in Kahn antigen is an ivory colored friable dry powder, giving the chemical reactions for creatinine and creatine.<sup>9</sup> Kahn antigen dilution in slide tests gave less satisfactory results than in standard Kahn tests.<sup>10</sup> From a few recent observations it appears that Kahn antigen base wax in ten parts absolute ethyl alcohol in slide test emulsions may give better results in slide tests than standard Kahn antigen dilution.

Dr. Johns has kindly furnished us some of his antigen base and we have started a study of it. This wax, also from alcoholic extract of heart powder previously extracted with ether, is soft and yellow brown, much like the Kahn wax. It emulsifies readily in water and when some wax in ten parts absolute alcohol is emulsified in a minimal quantity of water the microscopic appearance is much like that of the Kahn wax. As the fluid evaporates, numerous small needle-like crystals precipitate out of it (see fig. 5).



When an ounce or more of standard Johns antigen base is concentrated to about 10 per cent lipid content, a white precipitate of large crystals appears quickly at room temperature. This does not occur with Kahn base. Johns wax in ten parts absolute alcohol in slide test emulsions did not give quite as good results in slide tests as did the Kahn wax.

An unexpected outcome was the slide test results with a wax obtained by vigorous two hour absolute alcohol extraction of heart powder, previously extracted with ether. The ether extraction was carried out as specified in the preparation of Kahn antigen. This wax in ten parts absolute ethyl alcohol did not give altogether satisfactory results in slide tests. The explanation may be that more adventitious material is extracted from ether washed powder with absolute alcohol in two hours vigorous shaking than in three days gentle extraction with 95 per cent alcohol.

### 3. ACETONE INSOLUBLE FRACTION OF ALCOHOLIC EXTRACT OF HEART POWDER

Plain alcoholic tissue extracts and alcoholic extracts of tissue previously extracted with ether or acetone and ether extracts of tissue, all contain a phosphatide fraction obtainable by precipitation in acetone. This phosphatide fraction was first reported by Noguchi<sup>11</sup> to be the most potent and specific portion of tissue extracts. Corroboration of the specificity and potency of the acetone insoluble fraction of tissue extract has been reported by Browning, Cruikshank and McKenzie (12), Neymann and Gager (13), Kolmer (14) (15), Kiss (16) and Kline (7).

The phosphatide fraction free of alcohol soluble, acetone soluble and ether insoluble adventitious substances may be obtained by precipitation for 15 minutes of concentrated absolute alcohol extract of heart powder in fifteen volumes of warm acetone 55° to 37°C.<sup>7,9</sup> This precipitate is coherent, dark brown, waxy (standard slide test wax) and when rubbed or shaken in about one hundred parts of water undergoes complete emulsification.

The wax in ten parts of absolute ethyl alcohol when emulsified in a minimum quantity of water as described above shows light gold elongated globules somewhat more dense and coherent than those of Kahn and Johns antigen. As the free fluid evaporates no precipitation of crystals or other material occurs (see fig. 5). Slide tests for syphilis with standard slide test antigen give thoroughly satisfactory results.

Slide test waxes prepared by allowing the precipitation in acetone to continue too long or at too low a temperature, although coherent and dark brown and grossly indistinguishable from standard slide test antigen, behave differently when emulsified in water than do standard slide test antigens. In a minimal quantity of water improperly prepared slide test antigens show in places, on microscopic examination, a feathery precipitate like that in plain alcoholic extract and in some such antigens small needle-like crystals also precipitate out. Even more striking is the appearance when the wax is emulsified in a small quantity of water. Here large round antigen globules are

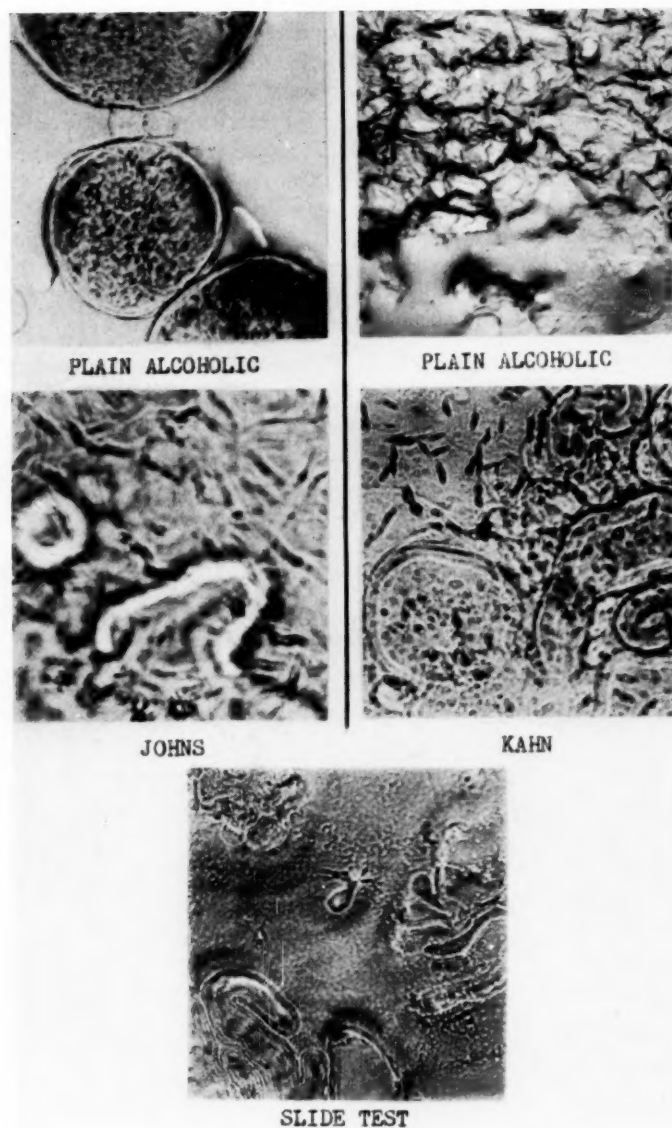
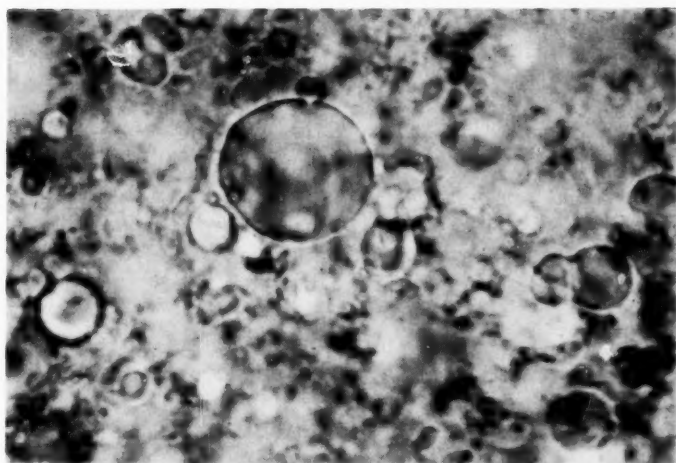
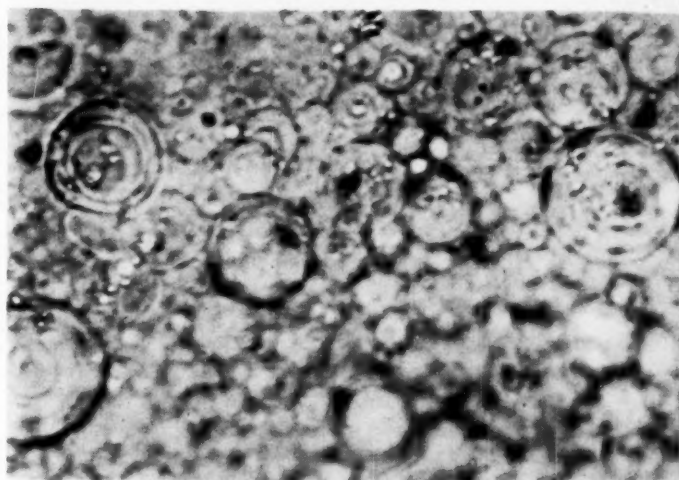


FIG. 5



STANDARD



IMPROPERLY PREPARED

FIG. 6

readily observed studded with numerous small granules in striking contrast to those of standard antigen which show no adventitious material (see fig. 6). Furthermore, improperly prepared slide test antigens give unsatisfactory results in slide tests for syphilis.

A decidedly unexpected result was the finding that the wax obtained by precipitation of concentrated standard Kahn antigen base in warm acetone not only gave unsatisfactory results in slide tests but also that on emulsification of the 10 per cent absolute alcohol solution in a minimal quantity of water, innumerable granules as well as some needle-like crystals precipitated out as the fluid evaporated. Likewise, a vigorous two hour absolute alcohol extract of heart powder previously extracted with ether as for the Kahn antigen, precipitated in warm acetone, yielded a wax that gave unsatisfactory results in slide tests.

These results indicate a striking difference in the quality of waxes obtained by precipitation in warm acetone of primary alcoholic extracts of heart powder and of those after preliminary ether extraction.

Additional observations relating to the acetone insoluble fraction of heart powder extracts are the following:

1. According to Erlandsen<sup>17</sup> there are four groups of phosphatides in heart muscle:

1. Monoamido-Monophosphatide (Lecithin-Cephalin group)
2. Monoamido-Diphosphatide (Cuorin)
3. Diamido-Monophosphatide
4. Diamido-Diphosphatide

2. Neymann and Gager<sup>13</sup> repeating the chemical separations described by Erlandsen, determined the antigenic properties of the various lipid fractions and found the most potent and most satisfactory fraction to be the diamido-monophosphatide group. Lecithin had much less antigenic property and Cephalin and Cuorin very much less.

Tentatively, the following criteria are offered for a standard antigen wax that could be used routinely in the various flocculation tests for syphilis if made up in about the same percentage as present in the antigens employed in those tests (8.75 per cent slide tests, 1.65 per cent Kahn tests, 0.85 per cent Johns test, etc.):

*Antigen wax free of adventitious substances*

1. Coherent dark brown?
2. Acetone insoluble.
3. Completely soluble in boiling ether.
4. Emulsifies readily in water.
5. When 10 per cent solution of wax in absolute alcohol is dispersed in a minimal quantity of water, no crystals precipitate out.
6. When 10 per cent solution of wax in absolute alcohol is dispersed in a

very small quantity of water, the globules are not highly refractile and do not show adventitious material upon them.

7. Gives results of maximum specificity in tests for syphilis.

## *II. Preparation of optimal antigen emulsions*

Given a standard antigen for syphilis, attempts should be made to determine a method or methods of preparing optimal antigen emulsions. This will require a study of the following:

1. Optimal quantity of standard antigen.
2. Optimal quantity of salt.
3. Optimal quantity of alcohol.
4. Optimal quantity of water.
5. Optimal quantity of cholesterin or other colloidal filler.
6. Optimal method of mixture of ingredients.
7. Optimal size of antigen particles.
8. Optimal shape of antigen particles.
9. Optimal stability of antigen particles.
10. Optimal temperature and time for ripening or increasing sensitivity of emulsion.

An emulsion of antigen in a small quantity of water or salt solution, contains small globular antigen particles requiring strongly positive sera for visible flocculation. With cholesterin or like substance present in the emulsion, the antigen particles are readily flocculated by even weakly positive syphilitic serum.

There is evidence that cholesterin or like substance increases the sensitivity of the emulsion by changing the antigen particles from globular form and variable size to larger needle or plate-like units with flat surfaces optimal for flocculation.

Emulsions generally employed in flocculation tests for syphilis are made by mixing salt solution with cholesterinized antigen solution. The resultant aggregates are composed of antigen lipid, cholesterin and other adventitious substances distributed more or less uniformly throughout the particles. The size and the shape of these particles are determined especially by the quantitative relationship of lipid and water, by the temperature of the ingredients and by the speed at which the mixture is made. In emulsions chemically identical for instance the particles may vary from less than one micron in diameter, as in the Kahn antigen dilution, when cholesterinized slide test antigen and abundant salt solution are quickly mixed, to large needle-like particles sixty micra or more in length, as in the Müller antigen emulsion, when cold cholesterinized slide test antigen is slowly mixed with very little cold salt solution. The sensitivity of such emulsions, depending in considerable part upon the size and shape of the antigen particles is, therefore, unavoidably variable, and satisfactory results are obtained only by observing the strictest

precautions relating to the quantity and temperature of the ingredients, to the speed of the mixture and to the size of the mixing vials. Furthermore, because of secondary changes such emulsions are unstable and unsatisfactory for use several hours after preparation.

In contrast to such emulsions are those prepared for slide tests by first precipitating the cholesterol (from alcoholic solution) in water and subsequently coating the crystals with antigen. Cholesterol plates precipitated from alcoholic solution in a small or large quantity of warm or cold water or salt solution, by slow or rapid mixture, vary but little in size and shape. Upon this fact depends the greater uniformity in antigen particles and to a considerable extent the greater uniformity in sensitivity of these emulsions. Furthermore, such antigen particles averaging about  $5 \times 3 \times 1$  micra are flat, plate-like structures optimal for flocculation and retain their antigenic power undiminished for 48 hours.

Concerning the optimal method of mixture of ingredients, there is some evidence that emulsions prepared as for slide tests, but employing Kahn antigen base wax, give better results in slide tests than does standard Kahn antigen dilution in slide tests.

Another type of antigen emulsion is that employed in the Hecht colored ball test.<sup>18</sup> This contains no cholesterol and when the antigen is dispersed in a very small quantity of alkalized salt solution, good sized elongated globular particles result.

Further studies of particles of different size and shape containing or capable of holding antigen on their surfaces may reveal those best for flocculation.

Concerning the "ripening" of antigen emulsions by heating above room temperature a short time, it is possible that it results in some swelling of the cholesterol in colloidal dispersion and makes the emulsion slightly more sensitive than when first prepared. This is probably a better method than "ripening" the emulsion for a short period at variable room temperature.

### *III. The determination of optimal test conditions*

These studies would include:

- A. Optimal antigen emulsion for diagnosis of syphilis.
- B. Optimal antigen emulsion for exclusion of syphilis.
- C. Optimal proportions of serum and antigen emulsion.
- D. Optimal state of serum; unheated (?); Heated at 56°C. (?).
- E. Optimal method of mixture of serum and emulsions. (In test tubes, on slides) (Rotation, shaking, centrifugation).
- F. Optimal temperature for carrying out tests.
- G. Optimal duration of tests.
- H. Optimal method of reading results, naked eye, low magnification (hand lens), magnification of 100 times or more (microscope).

Perhaps the first step advisable in attempting to standardize flocculation tests



for syphilis would be to make careful physical, chemical and biological studies of the antigens now in use and of others also with the objective of securing the most potent fraction in heart powder extract, free of adventitious substances to use as a base in the preparation of antigen emulsions. At this time the base could be used in the various flocculation tests in about the same concentration as in the present antigens employed (8.75 per cent slide test, 1.65 per cent Kahn tests, 0.85 per cent Johns test).

With a standard antigen free of adventitious substances and proper quantities of cholesterin, Balsam of Tolu, Sitosterol or other substance to increase the size and change the form of the antigen particles in emulsions, and with optimal test conditions, it may be possible to obtain maximum specificity, maximum sensitivity and greater uniformity of results in tests for syphilis than previously.

I wish to acknowledge the excellent technical assistance in the preparation of various antigens of Mrs. Dorothy Lloyd.

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# CONCERNING THE TITRATION OF ANTIGENS FOR COMPLEMENT FIXATION TESTS WITH SPECIAL REFERENCE TO SYPHILIS\*

JOHN A. KOLMER

WITH THE ASSISTANCE OF CAROLA E. RICHTER AND ELSA R. LYNCH

*From the Department of Bacteriology and Immunology, Temple University School  
of Medicine, Philadelphia*

Almost as important as the method of preparing antigen for the Wassermann or other complement-fixation tests, is the method employed for determining the optimum or best amount to employ for eliciting the maximum degree of sensitiveness consistent with specificity.

As is well known in various antigen-antibody reactions *in vitro* and especially in precipitation or flocculation tests, a fine quantitative adjustment of antigen and antibody is required for eliciting the maximum degrees of reaction since an excessively large amount of antigen or antibody may give weaker reactions than smaller amounts, constituting what is familiarly known as wanting or prezone reactions.

The oldest method consists in determining the anticomplementary unit of antigen and employing a fraction of this amount (usually one-fourth) for conducting complement fixation tests, on the assumption that it will supply the optimum amount free of anticomplementary or non-specific effects and yield specific reactions of maximum sensitiveness.

But as shown twenty years ago by L'Esperance and Coca<sup>1</sup> and Ottenberg,<sup>2</sup> amounts of antigen beyond a certain point in the Wassermann test may yield weak or falsely negative prezone reactions so that the optimum or best amount to employ may not be by any means the larger amounts and especially in tests where the complement is titrated in the presence of antigen.

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In my experience<sup>3</sup> this was found particularly true of occasional antigens more anticomplementary than usual requiring the use of relatively large amounts of complement and suggesting that some constituent of the serum, presumably the proteins, may interfere with complement fixation. But it may occur with antigens of very low anticomplementary activity, since I observed<sup>4</sup> that my cholesterolized and lecithinized extract (C.L. antigen) in the very large dose of 0.5 cc. of 1:10 dilution gave only a + reaction with 0.05 cc. of syphilitic serum, whereas + + + + reactions were observed with 0.5 cc. of 1:80 to 1:1280 dilutions. In bacterial complement fixation tests the phenomenon was not in evidence but in complement fixation tests for the identification and differentiation of blood stains, these prezone reactions were found even more pronounced than in the Wassermann reaction. For example, in one experiment 0.5 cc. of 1:100 dilution of sheep blood antigen gave a negative reaction with 0.005 cc. of antisheep serum whereas + + + + reactions were observed with 0.5 cc. of 1:400 to 1:2000 dilutions of antigen.

Furthermore, the amount of antibody present in the serum employed for the titration of antigens is likewise a factor of importance and in titrating antigen for the Wassermann test, it has been the practice to use only strongly positive sera with an endeavor to overcome variations in amounts of antibody by employing mixtures of these. Since serum is used in amounts from 0.2 to 0.005 cc. in my quantitative test<sup>5</sup> I have employed 0.05 cc. (0.5 cc. of 1:10 dilution) for titrating antigen in order to avoid the error due to the use of excessively large amounts of antibody.

The technic for determining the optimal amount of antigen to employ in the Wassermann and other complement fixation tests is, therefore, not as simple as pioneers in this field originally believed and as stated by Ottenberg (2), in relation to the Wassermann test, this should be the amount which gives a + + + + reaction with the smallest amount of syphilitic serum.

In 1922 I therefore proposed in the technic of my new complement fixation test for syphilis,<sup>6</sup> a method for determining the antigenic unit of cholesterolized and lecithinized antigen (C.L.)

by using 0.5 cc. of 1:300 to 1:4000 dilutions with 0.05 cc. of a mixture of strongly positive sera as these amounts of antigen and antibody were found not to yield prezone reactions. Since the antigen is very low in anticomplementary activity (usually 0.5 cc. of about 1:6) I proposed using it in a dose of 10 antigenic units (usually 0.5 cc. of 1:240 to 1:320) because it was found<sup>a</sup> to yield complement fixation reactions of maximum sensitiveness in doses of 5 to 15 units. Furthermore, this dose was observed to be usually 40 or more times less than the anticomplementary or non-specific dose and purposely large enough for the detection of small amounts of antibody on the basis of the well known phenomenon in complement fixation that within certain limits a larger amount of antigen is required for the detection of small amounts of antibody in weakly positive sera than required for the detection of large amounts of antibody.

Recently, however, Boerner and Lukens<sup>7</sup> have proposed a modification of this method for determining the optimal dose of antigen for the Wassermann test, by titrating it not only in six different dilutions, but with at least five amounts of positive serum instead of one with each amount of antigen in order to detect approximately the smallest amount of antigen giving ++++ reactions with approximately the smallest amount of serum, as the optimal amount of antigen to employ in the complement fixation test for syphilis. According to this method it is stated that the titration can be done with a single positive serum as well as with a mixture of sera and with serum containing small as well as large amounts of antibody. In view of the correctness of the principles involved, we have thought it advisable to compare the results with my method of titrating antigens not only for the Wassermann, but likewise for other complement fixation tests as well, and the results are briefly summarized herein.

#### TITRATION OF ANTIGEN FOR THE WASSERMANN TEST

In conducting antigenic titrations by the method of Boerner and Lukens, the extracts were used in 0.5 cc. of 1:80, 1:160, 1:320, 1:640, 1:1280 and 1:2560 dilutions with 0.1, 0.05, 0.025,

0.0125 and 0.005 cc. of positive serum, the hemolytic system, primary and secondary incubation, method of diluting antigen, etc., being exactly the same as in my method.

This set-up and the results of a titration of my new cholesterolized and lecithinized extract re-enforced with acetone insoluble lipoids<sup>8</sup> with strongly, moderately and weakly positive syphilitic sera and a mixture of these are shown in table 1.

TABLE 1  
TITRATION WITH C.L. ANTIGEN RE-ENFORCED WITH LIPOIDS

SERA	AMOUNTS SERUM	REACTIONS WITH 0.5 CC. DILUTIONS OF ANTIGEN					
		1:80	1:160	1:320	1:640	1:1280	1:2560
Strongly posi- tive	cc.						
	0.005	-	-	-	-	+	-
	0.0125	-	-	+	++++	++++	++
	0.025	+	+	++++	++++	++++	+++
	0.05	++++	++++	++++	++++	++++	++++
	0.1	++++	++++	++++	++++	++++	++++
Moderately positive	0.005	-	-	-	-	-	-
	0.0125	-	-	-	-	-	-
	0.025	-	-	-	-	+	-
	0.05	-	-	-	++	++++	+
	0.1	-	-	+++	++++	++++	++++
Weakly posi- tive	0.005	-	-	-	-	-	-
	0.0125	-	-	-	-	-	-
	0.025	-	-	-	-	-	-
	0.05	-	-	-	+	++	+
	0.1	-	-	-	++	++++	++
Mixture of the three	0.005	-	-	-	-	-	-
	0.0125	-	-	-	-	-	-
	0.05	-	-	+++	++++	++++	+++
	0.1	+	++++	++++	++++	++++	++++

It will be noted that the optimum ++++ reactions occurred with the 1:640 and 1:1280 dilutions of antigen and 0.0125 cc. of strongly positive serum; with 1:1280 antigen and 0.05 cc. of moderately positive serum; with 1:1280 antigen and 0.1 cc. of weakly positive serum and with 1:640 and 1:1280 antigen with 0.05 cc. of a mixture of equal parts of these sera. In other words

the optimum dilutions of antigen were not exactly the same with the varying amounts of antibody but according to Boerner and Lukens the optimum dose to employ would be 0.5 cc. of 1:640 dilution since they state that "when the optimum dose falls between two dilutions it appears safer to favor the stronger dilution due to the fact that there is very often a precipitous drop in the fixability of the higher dilutions."

When titrated by my method with 0.05 cc. of the same strongly

TABLE 2  
TITRATIONS OF PLAIN C.L. ANTIGEN

SERA	AMOUNTS SERUM	REACTIONS WITH 0.5 CC. ANTIGEN DILUTIONS					
		1:80	1:160	1:320	1:640	1:1280	1:2560
Strongly positive	cc.						
	0.005	—	—	—	—	—	—
	0.0125	—	—	++++	++++	++++	+
	0.025	+	++	++++	++++	++++	+++
	0.05	+++	++++	++++	++++	++++	++++
	0.1	++++	++++	++++	++++	++++	++++
Moderately positive	0.005	—	—	—	—	—	—
	0.0125	—	—	—	—	—	—
	0.025	—	—	—	+	+	—
	0.05	—	++	++++	++++	++++	+
	0.1	+	+++	++++	++++	++++	+++
Weakly positive	0.005	—	—	—	—	—	—
	0.0125	—	—	—	—	—	—
	0.025	—	—	—	+	—	—
	0.05	—	+	++++	++++	+++	+
	0.1	—	++	++++	++++	++++	++

positive serum the antigenic unit was 0.5 cc. of 1:12,000 and since this antigen is used in a dose of 20 antigenic units, equivalent in this instance to 0.5 cc. of 1:600, the dose by my method was quite close to that arrived at by the Boerner and Lukens titration. The anticomplementary unit was 0.5 cc. of 1:8 or from 75 to 80 times less than the optimum dose by both methods.

The results of a titration with plain C.L. antigen are shown in table 2.

Here it will be observed that the range of optimum antigenic



activity was from 0.5 cc. of 1:320 to 1:1280 and according to Boerner and Lukens the middle of the range, or 0.5 cc. of 1:640, would be the proper dose to employ.

When titrated by my method with 0.05 cc. of the same strongly positive serum the antigenic unit was 0.5 cc. of 1:4200 and since this plain C.L. antigen is used in dose of 10 units, equivalent in this instance to 0.5 cc. of 1:420, it will be observed that this dose was similar to the optimum amount arrived at by the Boerner and Lukens method and as the anticomplementary unit of this extract was 0.5 cc. of 1:6, the doses arrived at by both methods were from 70 to 100 times less.

The results of titrations by both methods of additional plain and re-enforced C.L. antigens, as well as of plain and cholesterolized (0.4 per cent) alcoholic extracts of beef heart and acetone insoluble lipoids (Noguchi), are summarized in table 3. It shows the smallest amounts of strongly, moderately and weakly positive sera which gave + + + + reactions with 0.5 cc. of the highest dilutions of the respective antigens and the optimum doses by the method of Boerner and Lukens as well as the antigenic units determined by titration with 0.05 cc. of mixtures of strongly positive sera in my method.

Since the dose by my method is 10 antigenic units except in the case of C.L. antigen re-enforced with lipoids in which case 20 units are used, it will be noted that the optimum dose of each antigen by the method of Boerner and Lukens has been closely similar to the doses employed by me and a few comparative complement fixation tests with C.L. antigens used in the optimum doses of Boerner and Lukens and the doses according to my method have given very similar results as shown in table 4.

In other words, the complement fixation reactions with 24 syphilitic sera tested with plain and re-enforced C.L. antigens in doses of 0.5 cc. of 1:320 and 1:640 according to titrations by the Boerner-Lukens method gave very similar reactions as the same antigens used in doses of 0.5 cc. of 200, 1:300 and 1:400 as determined by my method and indicating that the optimal range may be anywhere between 1:200 and 1:640 in so far as C.L.



TABLE 3  
SUMMARY OF RESULTS OF TITRATIONS OF VARIOUS ANTIGENS BY THE METHODS OF BOERNER AND LUKENS AND KOLMER

ANTIGENS	STRONGLY POSITIVE SERUM		MODERATELY POSITIVE SERUM		WEAKLY POSITIVE SERUM		KOLMER UNIT	DOSE	
	Smallest amount*	Antigen†	Smallest amount*	Antigen†	Smallest amount*	Antigen†		Boerner-Lukens	Kolmer
C.L. plain.....	0.0125	320; 640; 1280	0.025	640	0.05	320; 640	1:4000	1:640	1:400
	0.0125	640; 1280	0.05	640; 1280	0.05	640	1:4200	1:640	1:420
	0.0125	160; 320	0.05	160; 320; 640	0.05	320	1:3000	1:320	1:300
C.L. re-enforced.....	0.0125	160; 320; 640	0.025	320; 640	0.05	320; 640	1:6000	1:320	1:300
	0.0125	160; 320; 640	0.025	320; 640	0.05	320	1:6000	1:320	1:300
	0.0125	160; 320; 640	0.025	320; 640	0.025	320; 640	1:7000	1:320	1:350
	0.0125	640; 1280	0.025	1280	0.5	1280	1:16,000	1:1280	1:800
Cholest. alc. ext.....	0.0125	320; 640	0.05	320; 640	0.1	640	1:3000	1:320	1:300
	0.025	320; 640	—	—	—	—	1:3000	1:320	1:300
Plain alc. ext.....	0.025	320; 640; 1280	0.05	640	0.1	640	1:4000	1:640	1:400
	0.05	320; 640	—	—	—	—	1:2000	1:320	1:200
Acet. insol. lipoids.....	0.05	320; 640	0.05	320; 640	0.1	320	1:2000	1:320	1:200
	0.05	160; 320	—	—	—	—	1:1800	1:160	1:180

\* Giving +++ reactions by Boerner-Lukens method.

† Highest dilutions in dose of 0.5 cc. giving +++ reactions by Boerner-Lukens method.

antigens are concerned when employed in the complement fixation test for syphilis.

An examination of table 3 also shows that while the optimum doses of antigen as determined by the method of Boerner and Lukens are not exactly the same with strongly, moderately and

TABLE 4  
COMPARATIVE COMPLEMENT FIXATION REACTIONS WITH ANTIGENS USED IN OPTIMUM DOSES

ANTIGENS	BOERNER AND LUKENS		KOLMER	
	Antigen dose, 0.5 cc.	Reactions	Antigen dose, 0.5 cc.	Reactions
C.L. plain	1:640	44441	1:300	44441
	1:640	43----	1:300	44----
	1:640	1-----	1:300	3-----
	1:640	44443	1:300	44443
	1:640	4444-	1:300	44442
	1:640	4442-	1:300	4443-
	1:320	4443-	1:200	4442-
	1:320	43----	1:200	41----
	1:320	2-----	1:200	2-----
	1:320	44444	1:200	44443
	1:320	44443	1:200	44443
	1:320	44442	1:200	44441
C.L. re-enforced	1:640	44431	1:400	44431
	1:640	42----	1:400	42----
	1:640	2-----	1:400	2-----
	1:640	44443	1:400	44443
	1:640	44442	1:400	44442
	1:640	44432	1:400	44431
	1:640	4443-	1:300	4442-
	1:640	43----	1:300	43----
	1:640	1-----	1:300	1-----
	1:640	44443	1:300	44443
	1:640	4444-	1:300	4444-
	1:640	4443-	1:300	4443-

weakly positive sera, they are approximately the same and that a distinct advantage of the method is that most any positive serum may be employed for the titration (preferably one containing fairly large amounts of antibody) without the necessity of using mixtures. With C.L. antigen it will be noted that the optimum range has been observed with 0.0125 cc. of strongly

positive serum and usually 0.025 to 0.05 cc. of moderately and weakly positive sera.

One very important question arises as to whether or not different kinds of antigens used in their optimum doses according to titration by the method of Boerner and Lukens give similar complement fixation reactions in syphilis.

For example, the optimum dose of a plain C.L. antigen was 0.5 cc. of 1:200; the optimum dose of a C.L. antigen re-enforced

TABLE 5  
COMPARATIVE KOLMER COMPLEMENT FIXATION TESTS WITH THREE DIFFERENT ANTIGENS USED IN OPTIMUM DOSES (BOERNER AND LUKENS)

SERUM	C.L. PLAIN. DOSE: 0.5 cc. OF 1:200	C.L. RE-ENFORCED. DOSE: 0.5 cc. OF 1:640	CHOLEST. ALC. EXT. BEEF HEART. DOSE: 0.5 cc. OF 1:320
1	2----	3----	1----
2	1----	2----	2----
3	42---	441--	43---
4	442--	4441-	44---
5	41---	43---	4-----
6	444--	4442-	431--
7	4-----	43---	41---
8	4-----	43---	42---
9	444--	4442-	441--
10	4444-	4444-	4444-
11	44441	44443	44441
12	4444-	44442	4444-

\* 4 = +++++; 3 = ++++, etc.

with lipoids was 0.5 cc. of 1:640, while the optimum dose of an alcoholic extract of beef heart re-enforced with 0.4 per cent cholesterol was 0.5 cc. of 1:320. The results observed with 12 syphilitic sera tested simultaneously with all three antigens in these doses are shown in table 5. Each serum was used in amounts of 0.2, 0.1, 0.05, 0.025 and 0.005 cc. according to the technic of my quantitative test.

It will be observed that while the optimum dose of a C.L. antigen re-enforced with lipoids was 0.5 cc. of 1:640 it gave somewhat more sensitive reactions than plain C.L. in dose of 0.5 cc. of 1:200 or cholesterolized alcoholic extract of beef heart in dose of 0.5 cc. of 1:320.

These results indicate therefore that antigens employed in optimum doses according to the titration method of Boerner and Lukens do not necessarily yield identical results in Wassermann tests with syphilitic sera. In 1922 Miss Trist and I observed similar results<sup>9</sup> in a comparative study of eight different antigens in tests employing each in a dose of 2 or 5 antigenic units, finding that extracts re-enforced with 0.2 to 0.4 cholesterol gave more complement fixation than plain extracts.

In other words, one antigen used in its optimum dose according to titration may not yield as sensitive Wassermann reactions as a second antigen used in its optimum dose and this fact requires emphasis to correct any impression to the effect that it

TABLE 6  
TITRATION OF GONOCOCCUS ANTIGEN

AMOUNTS SERUM	REACTIONS WITH 0.5 CC. ANTIGEN DILUTIONS					
	1:16	1:32	1:64	1:128	1:256	1:512
cc.						
0.005	++	+	-	-	-	-
0.0125	++++	++++	+++	++	-	-
0.025	++++	++++	++++	+++	+	-
0.05	++++	++++	++++	++++	+	-
0.1	++++	++++	++++	++++	++	-

makes little or no difference what kind of antigen is employed as long as it is used in its optimum dose. But, on the other hand, the method of Boerner and Lukens for determining the optimum dose of antigen greatly reduces the differences in sensitiveness of Wassermann reactions with different antigens and constitutes an important advantage and improvement over usual methods including my own.

#### TITRATION OF ANTIGEN FOR BACTERIAL COMPLEMENT FIXATION TESTS

In my experience<sup>10</sup> bacterial antigens do not appear as likely to give prezone reactions as tissue extracts and the most sensitive complement fixation reactions have uniformly occurred with the larger amounts of antigen free of non-specific effects corresponding

to about one-fourth of the anticomplementary unit. This was found true of tuberculosis, gonococcus and *Br. abortus* antigens used in amounts corresponding to one-half to one-tenth of their anticomplementary units tested with varying amounts of the respective immune sera.\* That is to say, with bacterial suspensions I have found that "the larger the amounts of immune sera employed, the stronger were the reactions with small amounts of antigen, but *when the amount of antibody was small as in the higher dilutions of immune sera, the degree of complement fixation was in direct proportion to the amount of antigen employed.* In other words when the amount of antibody in the serum is small as it usually is in human tuberculosis and gonococcus infections, the sensitiveness of complement fixation tests is increased by using the largest allowable amount of antigen."

Similar results have been observed with bacterial antigens titrated according to the principles of the method of Boerner and Lukens which were exactly the same as employed by me.

The anticomplementary unit of a gonococcus antigen prepared according to the method of Price was 0.5 cc. of 1:8. It was used in the amounts shown in table 6 corresponding to  $\frac{1}{2}$ ,  $\frac{1}{4}$ ,  $\frac{1}{8}$ ,  $\frac{1}{16}$ ,  $\frac{1}{32}$  and  $\frac{1}{64}$  of this unit with the same amounts of antibody serum as employed in the titration of antigen for the Wassermann test.

Antigens of *B. typhosus*, *Br. abortus* and *B. tuberculosis* were anticomplementary in 0.5 cc. undiluted and were used in the same fractions varying from  $\frac{1}{2}$  to  $\frac{1}{64}$  of this amount with their respective antibody sera as shown in tables 7, 8 and 9.

It will be observed that in every instance the degree of complement fixation was highest with the largest amounts of the different antigens and that prezone reactions did not occur. In other words, the optimum dose of bacterial antigens appears to

\* All of our antigens however, were heat killed suspensions in saline solution and it appears that prezone reactions in bacterial complement fixation tests may occur with antigens prepared by other methods. For example, Boerner and Stubbs (Jour. Amer. Vet. Assoc., 55: No. 4, 1924) have observed their occurrence with bacteria-free antigens of *Br. abortus*. These were not as anticomplementary or as antigenic as suspensions heated at 100°C. for three hours but were found to fix more complement in small than in large doses.

be the largest amount that can be safely employed free of anti-complementary or non-specific effects and in general terms this

TABLE 7  
TITRATION OF *B. TYPHOSUS* ANTIGEN

AMOUNTS SERUM	REACTIONS WITH 0.5 CC. ANTIGEN DILUTIONS					
	1:2	1:4	1:8	1:16	1:32	1:64
cc.						
0.005	++++	++++	+++	+	-	-
0.0125	++++	++++	++++	++++	+++	-
0.025	++++	++++	++++	++++	++++	++
0.05	++++	++++	++++	++++	++++	++++
0.1	++++	++++	++++	++++	++++	++++

TABLE 8  
TITRATION OF *BR. ABORTUS* ANTIGEN

AMOUNTS SERUM	REACTIONS WITH 0.5 CC. ANTIGEN DILUTIONS					
	1:2	1:4	1:8	1:16	1:32	1:64
cc.						
0.005	+	-	-	-	-	-
0.0125	++++	++++	++	+	-	-
0.025	++++	++++	+++	+	-	-
0.05	++++	++++	+++	+	-	-
0.1	++++	++++	+++	++	-	-

TABLE 9  
TITRATION OF *B. TUBERCULOSIS* ANTIGEN

AMOUNTS SERUM	REACTIONS WITH 0.5 CC. ANTIGEN DILUTIONS					
	1:2	1:4	1:8	1:16	1:32	1:64
cc.						
0.005	+	-	-	-	-	-
0.0125	+	-	-	-	-	-
0.025	++	+	-	-	-	-
0.05	+++	+	-	-	-	-
0.1	++++	+++	+	-	-	-

may be one-third to one-fourth of their anticomplementary units.

Of course this does not mean that the methods employed for the preparation of bacterial antigens is a matter of indifference. As is true of antigen for the Wassermann test, the method of



preparation is one of importance<sup>11</sup> since it appears highly probable that no one method is applicable to all bacteria since some antigens remarkably free of anticomplementary activity are likewise very low in antigenic sensitiveness. In other words, the best antigens are those low in anticomplementary activity but possessing a high degree of antigenic sensitiveness, the relation between the two being of prime importance and conveniently expressed as the "antigenic index."

An antigenic titration is always advisable but not always possible due to the lack of a suitable positive serum and, as previously stated, I use bacterial antigens in an amount corresponding to one-third to one-fourth of their anticomplementary units even if an antigen happens to be so good that this dose carries more than 10 antigenic units, since prezone reactions apparently do not occur and because the larger the dose employed within safe limits, the greater the chances of detecting small amounts of antibody.

#### TITRATION OF ANTIGEN FOR COMPLEMENT FIXATION TESTS FOR THE IDENTIFICATION OF SERA AND BLOOD STAIN

As previously stated antigens of sera and blood are particularly likely to give prezone reactions. In my complement fixation method for their identification and differentiation<sup>12</sup> they are titrated for anticomplementary activity and used in one-half to one-eighth the unit but the dose should never be more than 0.5 cc. of 1:100 to avoid these reactions. Furthermore, the rabbit-immune serum should contain sufficient antibody to give complete fixation in dose of 0.5 cc. of 1:200, and preferably higher, so that at least two units or 0.5 cc. of 1:100 may be used in order to avoid prezone reactions and the property of rabbit sera for giving non-specific reactions.

For example, an antigen of one part human blood hemolyzed in nine parts of distilled water and rendered isotonic was not anticomplementary in dose of 0.5 cc. of 1:2. But when used in dose of 0.5 cc. of 1:10 with amounts of rabbit anti-human serum varying from 0.5 cc. of 1:100 to 1:1600, prezone reactions occurred as shown in table 10, whereas with doses of antigen varying from 0.5 cc. of 1:100 to 1:1200 these did not occur.



Therefore, in complement fixation tests according to my method for the identification and differentiation of sera, blood stains, meats and such substances, the optimum dose of antigen should be not more than 0.5 cc. of 1:100 and preferably 1:1000 and the rabbit immune serum should contain sufficient antibody to give complete fixation in dose of at least 0.5 cc. of 1:200 dilution.

TABLE 10  
PREZONE REACTIONS WITH AN ANTIGEN OF HUMAN BLOOD AND RABBIT ANTI-HUMAN SERUM

RABBIT IMMUNE SERUM (0.5 cc.)	REACTIONS WITH 0.5 CC. ANTIGEN DILUTIONS							
	1:10	1:100	1:200	1:300	1:400	1:600	1:800	1:1200
1:1600	—	—	—	—	—	—	—	—
1:1200	++	—	—	—	—	—	—	—
1:800	++++	+	—	—	—	—	—	—
1:600	++++	+++	+	—	—	—	—	—
1:400	++++	++++	++	+	—	—	—	—
1:300	++++	++++	++++	++	+	—	—	—
1:200	++	++++	++++	+++	++	+	—	—
1:100	+	++++	++++	++++	++++	+++	+++	+

#### OPTIMAL METHOD FOR TITRATING ANTIGEN FOR THE WASSERMANN TEST

Bacterial antigens therefore may be titrated for anticomplementary activity and one-third to one-fourth of the unit employed as the optimum dose for complement fixation tests.

Antigens of blood, sera, meat extracts, etc., should be titrated for anticomplementary activity but the optimum dose employed in complement fixation tests should never be more than 0.5 cc. of 1:100 and the rabbit-immune serum never used in a dose larger than 0.5 cc. of 1:100 in order to avoid prezone reactions and the non-specific fixing power of rabbit serum.

C.L. antigens for my complement fixation test for syphilis may be titrated as originally described and plain C.L. used in dose of 10 antigenic units and C.L. re-enforced with lipoids in dose of 20 units as the optimum amounts. But as several hundred antigens prepared and titrated during the past 14 years have never been hemolytic in dose of 0.5 cc. of 1:4 and since the anticomplementary units are usually around 0.5 cc. of 1:6 to 1:10 it is unnecessary to conduct titrations for hemolytic and anticomplementary activity. Titrations for antigenic activity, however, are always required and an optional or alternative method proposed by Boerner and Lukens and similar in principle to that employed by Eagle,<sup>13</sup> may be employed for determining the optimum dose as follows:

1. Prepare as 1:80 dilution of antigen by adding 0.1 cc. drop by drop with

shaking between each to 7.9 cc. of saline solution in a large test tube or small flask. Higher dilutions are then prepared as follows:

4 cc. of 1:80 + 4 cc. saline solution = 1:160  
 4 cc. of 1:160 + 4 cc. saline solution = 1:320  
 4 cc. of 1:320 + 4 cc. saline solution = 1:640  
 4 cc. of 1:640 + 4 cc. saline solution = 1:1280  
 4 cc. of 1:1280 + 4 cc. saline solution = 1:2560

2. Arrange 5 rows of test tubes with 6 in each row. In the *first* tube of each row place 0.5 cc. of antigen 1:80; in the *second* tube of each row 0.5 cc. of antigen 1:160; in the *third* tube 0.5 cc. of 1:320; in the *fourth* 0.5 cc. of 1:640; in the *fifth* 0.5 cc. of 1:1280 and in the *sixth* 0.5 cc. of 1:2560.

3. Heat 3 cc. of a moderately to strongly positive syphilitic serum in a water bath at 55°C. for 15 to 20 minutes and prepare 5 dilutions as follows in large test tubes.

1.0 cc. + 4.0 cc. saline = 1:5 (0.5 cc. carries 0.1 cc. serum)  
 0.5 cc. + 4.5 cc. saline = 1:10 (0.5 cc. carries 0.05 cc. serum)  
 0.5 cc. + 9.5 cc. saline = 1:20 (0.5 cc. carries 0.025 cc. serum)  
 2.0 cc. of 1:20 + 2.0 cc. saline = 1:40 (0.5 cc. carries 0.0125 cc. serum)  
 1.0 cc. of 1:20 + 4.0 cc. saline = 1:100 (0.5 cc. carries 0.005 cc. serum)

4. Add 0.5 cc. of 1:5 dilution to each of the six tubes of the first row; 0.5 cc. of 1:10 to each tube of the second row; 0.5 cc. of 1:20 to each tube of the third row; 0.5 cc. of 1:40 to each tube of the fourth row and 0.5 cc. of 1:100 to each tube of the fifth row.

5. Add 1 cc. of complement dilution carrying 2 full units to all tubes.

6. Put up a *serum control* carrying 0.5 cc. of 1:10 serum and 1 cc. of complement (2 full units), also a *hemolytic system control* carrying 0.5 cc. of saline solution and 1 cc. of complement (2 full units).

7. Shake the tubes gently and place in refrigerator at 6 to 8°C. for 15 to 18 hours, followed by water bath at 38°C. for 10 minutes.

8. Add 0.5 cc. of hemolysin (2 units) and 0.5 cc. of 2 per cent suspension of corpuscles to all tubes.

9. Mix thoroughly and place in a water bath at 38°C. for one hour; make readings. The serum and hemolytic system controls should show complete hemolysis.

10. Chart the results as per the following example observed with a strongly positive serum:

SERUM IN 0.5 cc.	ANTIGEN IN 0.5 cc. AMOUNTS					
	1:80	1:160	1:320	1:640	1:1280	1:2560
0.005	—	—	++	—	—	—
0.0125	—	+	++++	++++	++	+
0.025	+	++++	++++	++++	++++	+
0.05	+++	++++	++++	++++	++++	++
0.1	++++	++++	++++	++++	++++	+++

11. The optimum dose of antigen to employ in the main tests is the highest dilution giving a ++++ reaction with the smallest amount of serum. If two dilutions give ++++ reactions use the larger dose, as recommended by Boerner and Lukens, which in the above example would be 0.5 cc. of 1:320. If three dilutions give ++++ reactions, use the middle or average one as the optimum dose. For example, if ++++ reactions are observed with 0.5 cc. of 1:320, 1:640 and 1:1280 dilutions of antigen, the dose to employ would be 0.5 cc. of 1:640.

#### SUMMARY

1. An optional or alternative method by Boerner and Lukens for determining the optimum dose of antigen for the Kolmer complement fixation test for syphilis has been found satisfactory and the technic described.

2. In bacterial complement fixation tests the optimum dose of antigen is one-third to one-fourth of the anticomplementary unit as prezone reactions do not occur.

3. In complement fixation tests for the identification and differentiation of sera and blood stains prezone reactions with excessive amounts of antigen occur. According to the Kolmer technic the optimum dose of antigen should not exceed 0.5 cc. of a 1:100 dilution and the rabbit immune serum should contain sufficient antibody to give ++++ reactions in dose not exceeding 0.5 cc. of 1:100 dilution.

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## STORMY FERMENTATION OF MILK IN THE RECOGNITION OF *C. WELCHII* IN WOUNDS\*

C. LENORE ROBINSON AND W. D. STOVALL

*From the State Laboratory of Hygiene, Madison, Wisconsin*

Gas gangrene at times is difficult to diagnose early in its onset because of the indefinite clinical manifestations and the indecisive results of bacteriological studies within the first twenty-four hours. This indecision sometimes results in hasty action and bad practice.

For bacteriologists to admit indecisive results from cultural methods may come as a surprise, especially to those not engaged in the application of bacteriology to clinical diagnosis, and even to some bacteriologists who are familiar with anaerobic methods of cultivation in pure culture studies. The literature is replete with methods for the isolation and identification of *C. Welchii*. However, nearly all of them involve methods which can be used only in large laboratories equipped for all bacteriological procedures and even in these the time required to complete the study often negates its clinical value. For instance, Weinberg recommends a slide agglutination test for the identification of the various anaerobes found in gas gangrene. The sera for this test are not available in all laboratories and furthermore, more recent workers find several agglutinating strains of *C. Welchii* and some strains which agglutinate in any serum with difficulty; i.e., certain strains have a high degree of inagglutinability.

Most textbooks (Park and Williams, Zinsser, Ford) in speaking of the isolation of *C. Welchii* recommend the intravenous inocu-

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lation of rabbits with suspected material after which they are killed and incubated for 18 to 24 hours.

Such an animal will show at postmortem an abundant development of Gram positive, encapsulated, large non-motile bacilli and an extensive destruction of continuity of liver tissue—stormy fermentation. If this test could be always relied upon, it would be satisfactory although the use of animals would be a handicap for laboratories in small hospitals. Unfortunately nearly all, if not all, wounds in which the diagnosis of gas gangrene offers a problem contain a variety of organisms which for one reason or another not necessary to describe here complicate this test as a diagnostic aid and leave the result in doubt.

Wadsworth recommends that about 0.5 cc. of the material be inoculated into sterile tubes of milk and heated to 80°C. for fifteen minutes and that subcultures be made on other media. Since spores do not form in infected tissues, this temperature is likely to kill the vegetative forms and spoil the test.

Textbooks on clinical laboratory diagnosis are particularly vague on the methods to be used in the recognition of this infection.

One of the simplest tests described, and probably the one most used, is the stormy fermentation of milk with an odor of butyric acid by large Gram positive bacilli which are non-motile. We have used this method but not with entire satisfaction. In our experience the stormy fermentation of the milk, when the culture is made as usually described, is often delayed beyond the 24 hour incubation period and in certain cases does not occur beyond 48 hours; in such cultures sometimes the storminess of the fermentation is not characteristic, and when this feature of the test is confused the rest of it is not decisive. Since it is apparent that this test to be satisfactory must be reliable for the rapid development—18 to 24 hours incubation—of stormy fermentation from an inoculum which is always a mixed culture, we undertook the investigation, here reported, of several factors influencing the reliability of this simple method for the identification of *C. Welchii*.



## MATERIALS

Of the *C. Welchii* organisms used in these experiments, four strains were stock cultures, one was isolated from a post-operative wound which did not develop clinical symptoms although the organism was pathogenic for guinea pigs, one was isolated from the feces of the above-mentioned case, and the remaining four were isolated from cases of gas gangrene. The *B. coli* were isolated from wounds, associated with *C. Welchii*. One strain of streptococci was isolated from the feces of a patient, associated with *C. Welchii*, and was identified as *Streptococcus mitior*. Another, isolated from a wound, was *Streptococcus fecalis*.

The milk was fresh separated milk, tubed in 10 cc. portions and sterilized in the autoclave at 10 pounds pressure for fifteen minutes on two successive days. In the early experiments the milk was sterilized in the Arnold sterilizer for thirty minutes on three successive days. However, since the milk sterilized in the autoclave at 10 pounds gave equally good results and was simpler to prepare, this method of sterilization was used thereafter. This milk was again heated for a few minutes in boiling water just before inoculation to promote anaerobic conditions.

Muscle tissue was removed aseptically from freshly killed guinea pigs and added to the milk in the proportion of 0.7 to 0.8 gram to 10 cc. of milk. The blood also was from guinea pigs and was added to the milk in portions of 0.5 cc. and 1.0 cc. to 10 cc. of milk. Both the blood and fresh tissue were added to the milk after the milk had cooled just before inoculation. Tissue added to the milk before it was sterilized is referred to as sterilized tissue.

Powdered iron, reduced by the chemical action of hydrogen, was sterilized in small portions—1 to 2 grams—in test tubes, in the dry oven and kept tightly corked until needed. It was then added to the milk in the proportion of approximately 175 mgm. to 10 cc. of milk by means of a small platinum spoon. This iron may be stored for at least three to four months without appreciable loss of its effectiveness.

Inoculations of *C. Welchii* were made with the whey from 12–24 hour milk cultures, shaken with glass beads and filtered through cotton. Bacterial counts were made on these filtrates by means



of a hemocytometer. The counts ranged generally between four and six billion organisms per cubic centimeter. Serial dilutions of 1:10, 1:100, 1:1000, etc., were made in saline and 0.1 cc. portions of these suspensions were inoculated into milk tubes, ten tubes in each series.

The streptococci were grown 18 hours on blood serum, washed off in saline and used for the inoculations. *B. coli* was grown on plain agar and suspensions were made in the same way as with the streptococci.

#### EXPERIMENTAL WORK AND RESULTS

*Experiment 1.* In order to determine the comparative values of plain milk and milk containing actively reducing substances such as blood, tissue, and reduced iron, we have studied both the number of organisms required to produce a stormy fermentation of the milk and the type of fermentation obtained in each case. All cultures were made in series of ten tubes each inoculated with serial dilutions of the organisms as previously described. Table I shows the results obtained with a representative series of cultures. We found that stormy fermentation did not regularly occur in plain milk in eighteen hours incubation when the inoculum contained less than 450,000,000 organisms. In the same length of time milk containing 1 cc. of blood showed a moderately stormy fermentation with 1/1,000th of this inoculation. On the other hand, when iron was added to the milk, a definitely stormy fermentation resulted with 1/1,000th to 1/10,000th of this inoculation, while with fresh tissue added the same results were obtained with 1/10,000th or frequently 1/10,000,000th of the organisms required to ferment plain milk.

Figure 1 shows the variation in the character of the fermentation obtained in the cultures given in table I. The photographs were made at the end of eighteen hours incubation. The tubes containing fresh tissue (I), show an almost complete dispersion of the curd, and those containing iron (II) show a fine dispersion in contrast to the partial to slight disruption of the curd with blood (III) or plain milk (IV).

A repetition of the above experiment, using sterilized tissue, showed this to give better results than plain milk, but it was definitely inferior to fresh tissue both in the time required for the fermentation to take place and in the character of the resulting fermentation. Even the temperature reached in the deoxygenation of the milk interfered with the character of the fermentation so that it is preferable to add the tissue after the milk has cooled. Less than 0.6 gram of fresh tissue also gave an atypical fermentation and the curd was only partially dispersed by gas or the milk was only coagulated with little or no gas formation even when incubated 48-72 hours.

Obviously, plain milk is effective only when the number of organisms in the inoculum is large or when the inoculation is made with tissue from the wound. Blood and sterilized tissue are only slightly more effective and the less stormy character of the fermentation with these makes the interpretation of results more difficult than when iron or fresh tissue are added to the milk. For the remainder of our work we used only iron and fresh tissue.

In the earlier studies we used a vaseline seal for all tubes, but when comparative tests were made with and without vaseline, we found that its use with tubes containing fresh tissue or iron added only slight and inconstant advantages.

TABLE I  
INFLUENCE OF REDUCING SUBSTANCES ON TIME AND NUMBER OF ORGANISMS  
REQUIRED FOR THE STORMY FERMENTATION OF MILK BY C. WELCHII

NUMBER OF ORGANISMS	PLAIN MILK				MILK WITH 1 CC. BLOOD				MILK WITH IRON					MILK WITH FRESH TISSUE				
	18 hours	24 hours	36 hours	48 hours	18 hours	24 hours	36 hours	48 hours	12 hours	18 hours	24 hours	36 hours	48 hours	12 hours	18 hours	24 hours	36 hours	48 hours
450,000,000	+				+				+					+				
45,000,000	C	±			±	+			+					+				
4,500,000	-	-	C	C	±	±			-	+				+				
450,000	-	-	-	-	±	±			-	+				+				
45,000	-	-	-	-	-	±			-	-	+			+				
4,500	-	-	-	-	-	-	±		-	-	+			-	+			
450	-	-	-	-	-	-	C	C	-	-	-	+		-	+			
45	-	-	-	-	-	-	-	-	-	-	-	+		-	+			
4	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+, stormy fermentation.

±, moderately stormy fermentation.

C, coagulation with perhaps some gas formation.

-, no reaction.

In the remainder of our experiments no special means of securing anaerobiosis other than the addition of fresh tissue or iron to the milk were used.

*Experiment 2.* Marchal<sup>1</sup> states with reference to anaerobic bacilli that the dilution of the inoculum has no constant influence upon the resulting growth. We have made studies to determine if this applies to the fermentation of milk by C. Welchii. Table I shows readings made at 12, 18, 24, 36, and 48 hours in a representative set of cultures with fresh tissue and with iron.

It is observed that with fresh tissue added to the milk a stormy fermentation always results in 12 hours if the inoculum contains at least 450,000 organisms. Usually the same result is obtained with 45,000 or often fewer organisms. In 18 hours, 4,500 or frequently 25-50 organisms will give a stormy fermentation.

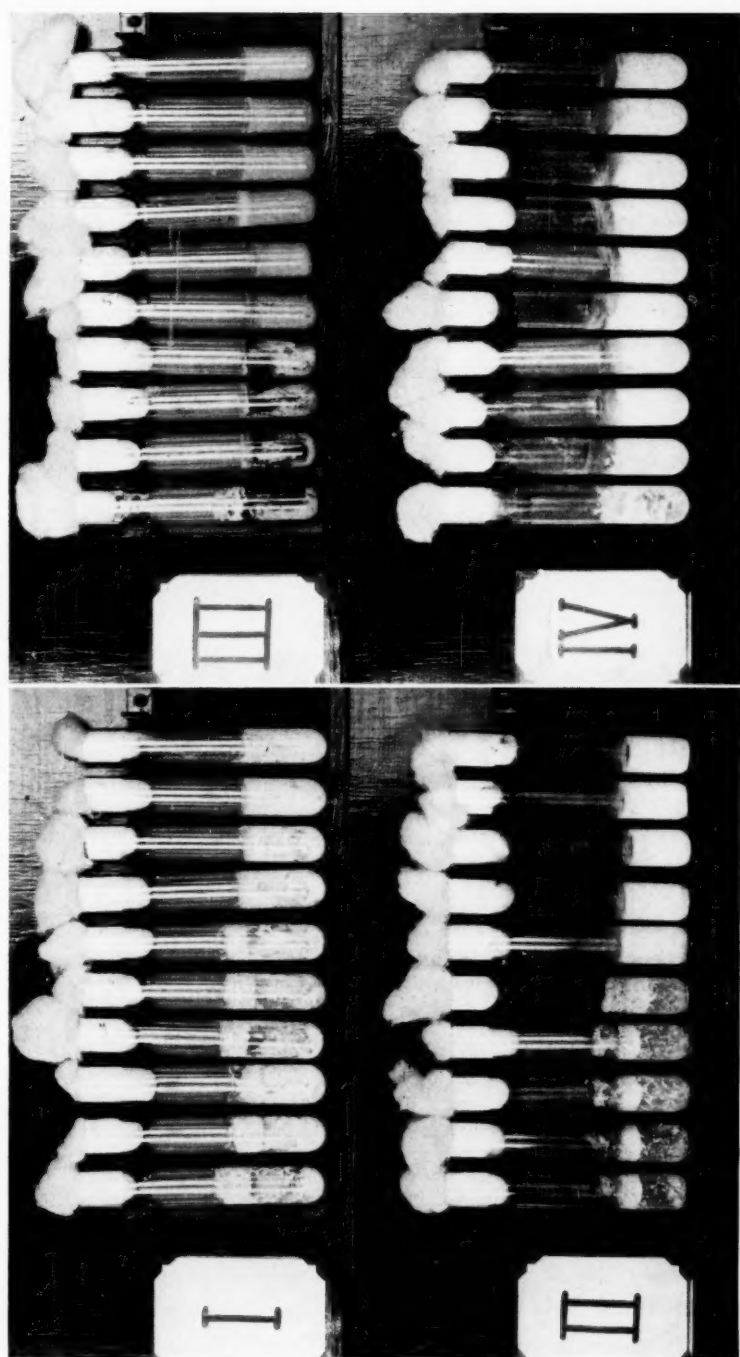


FIG. 1. INFLUENCE OF REDUCING SUBSTANCES ON THE CHARACTER OF MILK FERMENTATION BY *C. WELSHII*  
 I, milk-tissue; II, milk-iron; III, milk blood; IV, plain milk

In every case in this experiment we have had a stormy fermentation in 24 hours or less if the inoculum contained at least 450 organisms.

When iron was used, the fermentation took place more slowly. Readings at 12 hours usually showed fermentation when the inoculum contained at least 45,000,000 organisms; at 18 hours, with 450,000 or frequently 45,000 organisms; at 24 hours, with 4,500 or frequently 450 organisms; and at 36 hours, often with 45 organisms.

Fermentations, even with our largest inoculations, seldom occurred in less than nine to ten hours.

It is especially noteworthy that at the end of 36 hours the picture with iron is essentially the same as with fresh tissue, yet the time factor is much more important with the iron than with the fresh tissue. With tissue the picture is often complete at the end of 18 hours and there is seldom any change after 24 hours. On the other hand, when iron is used, the picture is seldom complete at the end of 24 hours and frequently there is a very definite change between 24 and 36 hours. Therefore, in diagnostic procedures when the size of the inoculation is uncertain and the time factor is important, the use of fresh tissue in milk is preferable to any other modification which we have tried.

*Experiment 3.* The above experiments have been dealing with pure cultures of *C. Welchii* where the conditions to a great degree can be controlled. To what extent these findings are modified by mixed cultures containing organisms quently associated with it in gas gangrene was therefore investigated. We studied especially two strains of streptococci and one of *B. coli*.

Cultures of *C. Welchii* in milk containing fresh tissue and iron were made as in the previous experiment and were then inoculated with constant numbers of streptococci. In each case control series were made using *C. Welchii* alone. Figure 2 shows the contrast between the fermentation of milk-tissue inoculated with *C. Welchii* alone (400,000,000 organisms in the first dilution) (I), and mixed with *Streptococcus fecalis* (200,000,000 organisms per tube) (II). When streptococci were present, stormy fermentation took place only in the first tube. Those tubes containing higher dilutions of *C. Welchii* showed coagulation only in the second and third tubes and no fermentation thereafter. This is in contrast to the stormy fermentation through eight tubes of the pure cultures of *C. Welchii*. Figure 3 shows a similar contrast when iron was substituted for the tissue. The *C. Welchii* alone (I) shows a fine dispersion of the curd and the mixture of *C. Welchii* and *Streptococcus fecalis* (II) shows a separation of the curd and whey but little or no dispersion of the curd. Thus we find that not only is fermentation distinctly inhibited but when it does take place, it is atypical unless very large numbers of *C. Welchii* are present.

When Gram stains were made from these mixed cultures, we found in the first tube of the series many *C. Welchii*. In successive tubes the number of these organisms rapidly diminished until in tubes four or five we found only streptococci.

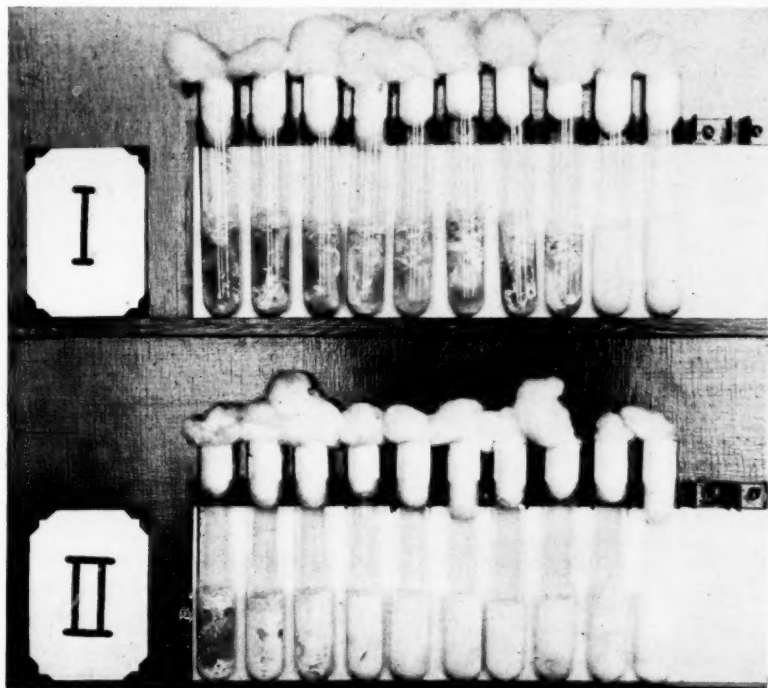


FIG. 2. INHIBITIVE ACTION OF STREPTOCOCCI ON *C. WELCHII* IN MILK-TISSUE CULTURES

I, *C. Welchii*; II, *C. Welchii* and streptococci

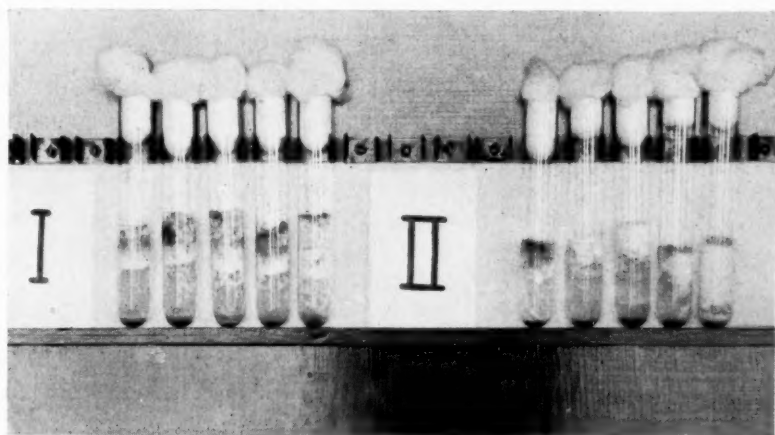
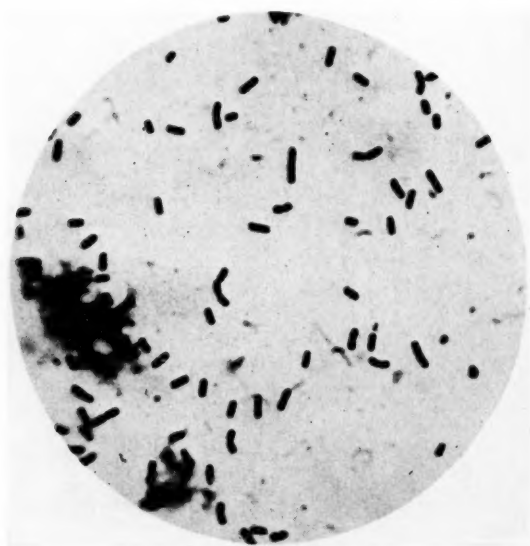
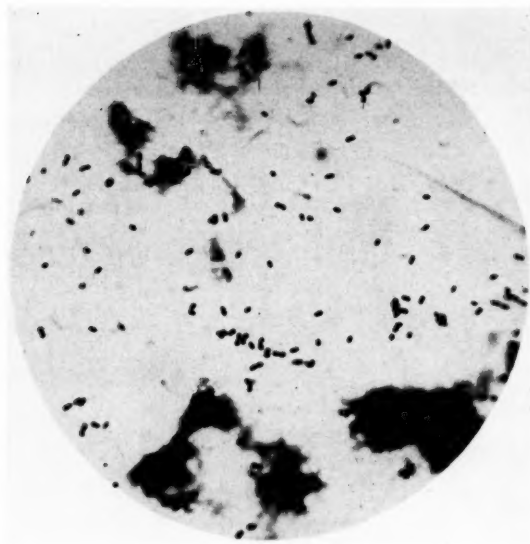


FIG. 3. INHIBITIVE ACTION OF STREPTOCOCCI ON *C. WELCHII* IN MILK-IRON CULTURES

I, *C. Welchii*; II, *C. Welchii* and streptococci



I



II

FIG. 4. MICROPHOTOGRAPH SHOWING OVERGROWTH OF *C. WELCHII*  
BY STREPTOCOCCI  
I, *C. Welchii* in pure culture; II, *C. Welchii* and streptococci



When this experiment was repeated, using 25,000,000 streptococci to each tube, the results were similar. However, definite inhibition began in the third rather than in the second tube. This would indicate that the inhibitive action of streptococci is influenced by the proportion of the two organisms in the inoculum.

*B. coli* did not show a very marked inhibition of the *C. Welchii* as demonstrated by smears; however, it did alter to some degree the stormy character of the fermentation.

#### DISCUSSION

In view of the variability observed in cultures made to determine the presence of *C. Welchii* in wounds and the scarcity of information to be found on the subject, we have undertaken to determine a method of modifying milk to improve its value for this purpose, the relationship of dosage to the time required for this organism to give a stormy fermentation of milk, and the effect of certain associated organisms upon the fermentation of milk by *C. Welchii*.

It had been noted in this laboratory that when fragments of tissue from infected wounds were introduced into the milk cultures, the results appeared to be more clear cut than when cultures were made from swabs contaminated with wound exudate. Tissue, either cooked or fresh, has been recommended by many workers for the culture of anaerobes. We have found that fresh tissue is superior to anything that we have used for promoting the fermentation of milk by *C. Welchii* since a distinctly stormy fermentation is produced with smaller numbers of organisms and in a shorter length of time than in plain milk, or blood and milk, and fermentation is produced in a shorter length of time than with iron and milk. Gates and Olitsky,<sup>2</sup> however, state that the reducing substance is present in extracts of kidney tissue and is relatively heat stable. It would seem from this that sterilized tissue would have approximately the same value as fresh tissue. We did not find this to be the case when we used muscle tissue of guinea pigs. While tissue sterilized in the milk gives better results than milk alone, fresh tissue is definitely more effective both in the promotion of fermentation and in the determination of its stormy character. Even the temperature reached in the

deoxygenation of the milk previous to inoculation interferes with the character of the fermentation so that it is preferable to add the tissue after the milk has cooled.

Owing to reports which have been made on the presence of *C. Welchii* in normal tissue, an objection may be raised to the use of unsterilized tissue in culturing exudates or other types of specimens for this organism. We have made many control cultures with the tissue used in these experiments and in routine cultures and have never had any fermentation. However, in routine cultures, we use both iron and fresh tissue and in addition we make control cultures of the tissue of each animal that is used. These precautions should prevent any error.

Following the recommendations of Hastings and McCoy<sup>3</sup> and Scott and Brandley,<sup>4</sup> we have studied the use of reduced iron and find it quite effective when the inoculation is fairly large or when the time factor is not especially important. It has an advantage over fresh tissue in that it can be sterilized and stored until needed. Also, the curd is very finely and completely dispersed, more so even than with fresh tissue, so that if the inoculation is sufficiently large to produce a reaction at all, it is easily recognized. The disadvantage in the use of iron for diagnosis is that it requires several hours longer for the reaction to take place than when fresh tissue is used and the influence of dosage is marked, the reaction being markedly delayed in the tubes inoculated with small numbers of organisms.

Since milk alone, or with blood added, gives typical reactions only when large numbers of organisms are used, these are not recommended for routine diagnostic cultures.

Among the organisms commonly associated with *C. Welchii* in wounds are streptococci and *B. coli*. We have often encountered considerable difficulty in separating *C. Welchii* from streptococci in cultures. Frequently when cultures in milk show numerous *C. Welchii* together with streptococci, subcultures show only streptococci. There is some difficulty noticed in separating *C. Welchii* from *B. coli* but there is not such a rapid disappearance of the *C. Welchii* from subcultures as is the case with streptococci. In these studies we have demonstrated, both macroscopically and

microscopically, a definite inhibition of the normal growth of *C. Welchii* in milk cultures by *Streptococcus mitior* and *Streptococcus fecalis*. This fact must be considered when interpreting the cultural reactions since these streptococci exert an inhibitive action on *C. Welchii*. Unless the inoculum contains a proportionately larger number of *C. Welchii*, the fermentation is prevented or is atypical in character.

*B. coli* shows some inhibitive action especially in altering the appearance of the fermentation.

RECOMMENDED PROCEDURE FOR THE LABORATORY IDENTIFICATION OF  
*C. WELCHII*

1. Make Gram and capsule stains and moist preparations and examine for large, non-motile, encapsulated, Gram-positive bacilli.
2. Place 3 tubes of milk in water and heat until water boils for 3-5 minutes.
3. Into one tube put approximately 175 mgm. of sterile iron.
4. Into each of two tubes put at least 0.7-0.8 gram fresh tissue. One of these serves as control.
5. Inoculate one tube of each kind of medium with the material to be examined. Swabs may be cultured directly into the medium.
6. The time required for a positive reaction will vary with the number of organisms in the inoculum. As soon as stormy fermentation is noted, make Gram stains and moist preparations to determine the presence of non-motile organisms having the morphology of *C. Welchii*. If these are present, a positive report is indicated. (We have not been able to demonstrate definite capsules in milk cultures with the Hiss' capsule stain.)

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## PHOTOGRAPHIC AND PHOTOMICROGRAPHIC TECHNIC\*

C. ALEXANDER HELLWIG

*From the Pathological Department, St. Francis Hospital, Wichita, Kansas*

In a recent Editorial of the Journal of the American Medical Association,<sup>1</sup> it was predicted that the hospital of the future will regard a photographic department as an economic and scientific necessity. While, at the present, research institutes and medical schools could not do satisfactory work without an elaborate illustration division staffed with artists and professional photographers, the non-teaching general hospitals have to do their photographic work under less favorable conditions.

I believe that the large hospital should, and eventually will, have a photographic department under the supervision of a biological photographer. It is evident, however, that the pathologist cannot wait, until the hospital boards decide to organize photographic departments. More than any other hospital division, does the laboratory feel the need for photographic records. For this reason I organized, four years ago, a photographic department in our hospital, without outside help. It is supervised by the pathologist and the technical work is done by laboratory technicians, though some day, I hope, a full time biological photographer will be in charge of this department.

The following notes on the organization of our photographic department, and our photographic and photomicrographic technique are not offered to professional photographers, but to pathologists who are in the same position as I am and who want accurate recording of clinical lesions and pathological findings. The procedures outlined are not original, but they are reduced to such simple and completely standardized maneuvers that anybody can with perfect regularity make good pictures.

We have two rooms, each measuring 12 x 20 feet. One is

\* Read before the Fifteenth Annual Convention of the American Society of Clinical Pathologists, Kansas City, Missouri, May 8-10, 1936. Received for publication, June 3, 1936.

reserved for clinical photography, the other, which is connected with a dark room, is for macrophotography of pathological specimens and for photomicrography.

Orders for photographs are accepted only from physicians and a special blank has to be filled out. The photographer enters in our journal the case number, name of patient and physician, lesion, and in every case also the technic of the photograph; i.e., lens, filter, light, extension of camera, plate or film, lens stop, time of exposure and of development. The photographic case number is marked on the negative and also on the finished prints. One print is filed in the photographic department, one other is attached to the patient's chart. There is a cross index of the photographs, according to the different organs.

#### PLATES, FILMS AND PRINTING PAPERS

Panchromatic plates are used exclusively for gross specimens and photomicrographs. We use Wratten M and Cramer Spectrum plates, 5 x 7 inches and 9 x 12 cm. which are developed in Green Safe Light. For clinical photographs and copies of x-ray films, Eastman Superspeed Portrait films 5 x 7 inches, are excellent. They are developed in red light.

Eastman process film or Cramer Supercontrast plate is the best negative material for copies of charts, drawings, typewritten and printed material. For lantern slides, we have Eastman Slow and Regular, and, for special contrast, Cramer Supercontrast lantern slides.

Prints are made by contact on glossy Azo F paper which comes in several grades, grade 1 for very dense, grade 5 for extremely flat negatives. They are developed in yellow light.

We use tray developing for all our material and our universal developer is good for plates, films, lantern slides and printing paper. We make up our own developer according to the formula given by W. G. MacCallum:<sup>2</sup>

#### STOCK SOLUTION

Dissolve in the order named:

Water at 52°C.....	1,420	cc.
Elon.....	7.1	gram
Sodium Sulphite.....	106	gram
Hydroquinone.....	28	gram
Sodium Carbonate.....	149	gram
Potassium Bromide.....	4	gram
then add:		
Methyl Alcohol.....	185	cc.

For use take stock solution 1 part, water 3 parts.



The development of plates takes about 3 minutes, that of lantern slides and printing paper 1½ minutes. After washing in water for a few seconds, the material is placed in fixing bath for 15 minutes. The fixing bath is made by dissolving Kodak Acid Fixing Powder in water. Plates and films are washed afterwards for 15 minutes, paper for one hour. The prints are dried on Ferrotype boards which are rubbed before use with Ferrolene solution to prevent sticking of the prints.

#### CLINICAL PHOTOGRAPHY

One of the narrow walls of our clinical photography room is covered with a Mazonite board, 6 x 8 feet. The smooth fawn-colored surface is an ideal background. A portable reflector, 8 feet high is set on one side of the patient to make the shadows lighter. The clinical camera is a 5 x 7 view camera on a heavy tripod. It has a double extension bellows, a ground glass back, and an anastigmat lens of 7½ inch focal length. An anastigmat lens is a necessity as a good clinical photograph should have microscopic detail. For focusing we use a photoflood Mazda light in a portable goose neck stand.

All our exposures are made with the Mazda Photoflash. It may be objected that the routine use of Photoflashes is too expensive. We found however, that the cost of 20¢ for one flash, is more than compensated by the saving of films, time, and energy of the photographer. Since using the photoflash method, it is extremely seldom that we have to repeat exposures. This is a very important factor, especially in taking pictures of operations, or in photographing very sick patients or small children who will not cooperate.

Our procedure in taking a clinical photograph is as follows: the patient is placed about 20 inches in front of the background and the photoflood light so directed as to provide the necessary illumination for focusing the lesion on the ground glass of the camera. The shutter and diaphragm of the lens are opened and the desired picture, in the right scale, is focused sharply on the ground glass by turning the knob which moves the bellows in and out. When focusing has been completed, the photoflood light is switched off, the diaphragm is closed to about f.32, the shutter is closed, the film holder placed in the back of the camera and the slide of the filmholder removed. Finally the shutter is opened and the photoflash lamp is pointed at the lesion so that an angle of 45 degrees is formed, care being taken that the light from the lamp does not strike the lens. When the switch on the handle of the flash lamp is pressed, the exposure is made. To complete the procedure, it is necessary only to close the shutter and remove the film holder.

#### X-RAY PRINTS AND LANTERN SLIDES

The following method of making x-ray reproductions was described by F. R. Harding,<sup>3</sup> photographer of the Children's Hospital in Boston and is far superior to our old method of using a regular x-ray illuminator. The ap-



paratus is simple and can be made by any carpenter. A "U" shaped frame has a  $\frac{1}{4}$  inch square groove cut in each upright and is to accommodate the glass "sandwich" which is a sheet of plain and a sheet of opal glass, 14 x 17 inches in size. In use, the opal glass side is placed toward the light. A set of black paper masks are used from 5 x 7 inches up, to block out the surplus space when using x-ray films smaller than the whole glass. A 500 watt bulb in a reflector is placed about 4 feet from the stand, to give even illumination over the whole opal glass.

We use the same 5 x 7 camera as for clinical photography with a focal length of  $7\frac{1}{2}$  inches. The camera is placed in front of the stand, then the glass sandwich is removed from the holder and the proper size mat is laid on the

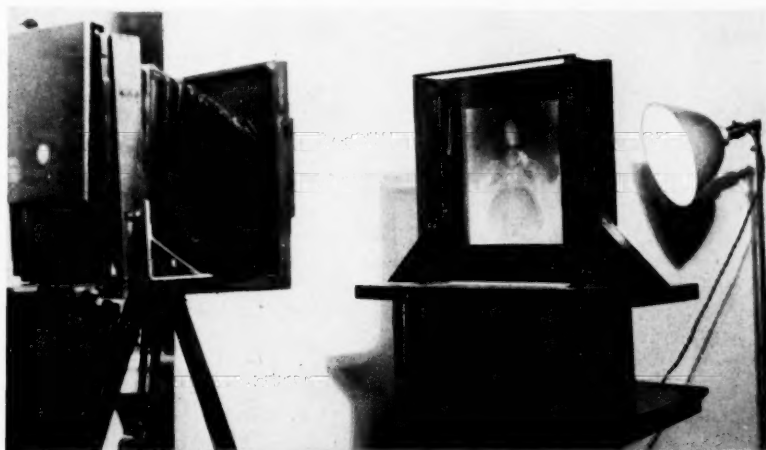


FIG. 1. REPRODUCTION OF X-RAY FILMS

The film is in the glass "sandwich" of the "U" stand. A 500 watt bulb in the goose neck stand provides even illumination.

opal glass. The x-ray is laid on top of this and the top or clear glass is lowered over it. The sandwich is then lowered in the grooved uprights of the stand and the light turned on. During the focusing and exposure, all other lights in the room are turned off to avoid reflections on the glass. The exposure is made on Eastman Superspeed Portrait Film. We stop the lens to f.24 and give an exposure that varies with the x-ray density from 2 to 8 seconds. The exposure is made by switching on and off the 500 watt light while the lens shutter is open.

The resulting films are printed on Azo F, grade 1 or 2, paper by contact. Prints and lantern slides made from these films retain all the detail of the original x-ray.

## PHOTOGRAPHY OF GROSS SPECIMENS

Our old method of immersing the specimen in water has been discarded and we use a set-up which was described by L. Schmidt,<sup>4</sup> head of the Illustration Division of the Rockefeller Institute. It is much cleaner, more convenient and gives much greater plasticity in the photograph. The wet specimen is

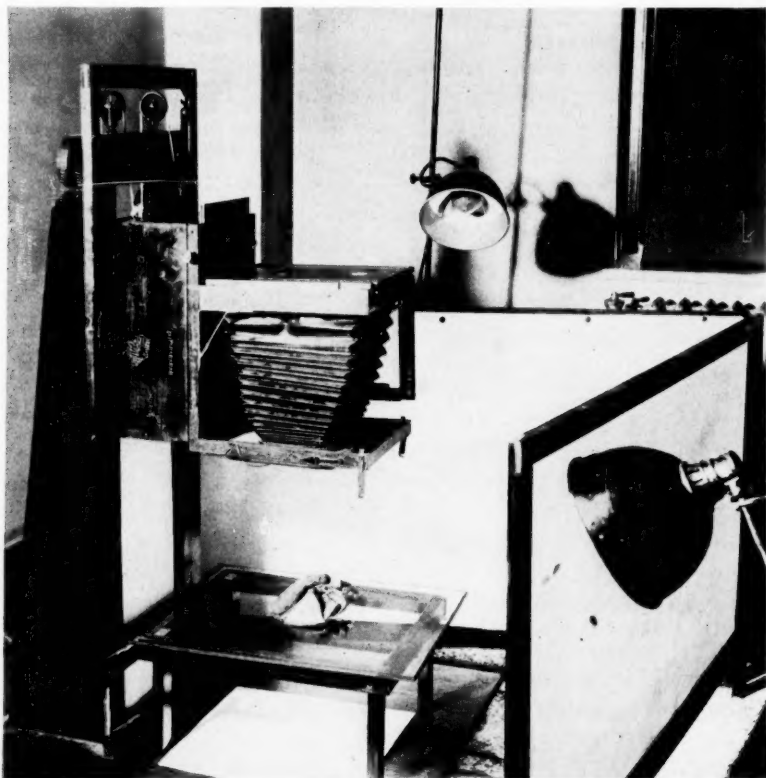


FIG. 2. SCHMIDT'S SET-UP FOR PHOTOGRAPHY OF GROSS SPECIMENS

The camera on the elevator unit runs in grooves of two uprights. A two-part screen is placed between light sources and specimen. The light in the back should be about 2 feet lower.

placed under a vertical camera and the two 500 watt bulbs which are used for lighting the specimen, are placed behind a two part screen covered with white curtain material. The camera lens and the specimen are thus protected from direct light and the pictures are well rounded, with clear detail in the shadows, yet free from sparkling highlights.

The stand consists of a platform mounted on rubber tired wheels. From this base rise uprights which are grooved at the inside. The camera, an 8 x 10 view camera with 50 cm. bellows extension, ground glass and a 15 cm. Tessar is mounted on a box which slides in the grooves of the two uprights. This elevator unit with the camera is counterbalanced by lead weights and is easily raised and lowered. A glass plate, 16 x 19 inches, for the specimen, rests on a support about one foot above the platform. Different shades of gray and black cardboard are placed on the base of the platform, until the most suitable one is found. If the lights are properly placed, there will be no shadow on the background.

The camera bed is marked for bellows length to produce full and one-half sizes. The camera is equipped with backs for different plate sizes, from 8 x 10 to 3½ x 4 inches.

The same apparatus is used in making copies of printed and type-written material. The book or sheet of paper to be photographed, is placed on the platform and is covered with a glass plate to obtain a flat surface. Lantern slides and enlargements are easily made with the same camera, by placing a light box with the negative under the camera.

Photomicrographs with a magnification from 1½× to 3× can be made, by placing the microscopic slide on the groundglass of the lightbox.

#### PHOTOMICROGRAPHY

There is no mystery in photomicrography and the apparatus does not have to be elaborate. However, to obtain good results, a few simple rules have to be followed.

The apparatus should be free from vibration. The source of light, condensers, microscope and camera should be capable of being centered into one axis and fixed in that position. The plane of the viewing glass must be parallel to that of the microscope stage. Provided these arrangements are fulfilled, the simplest apparatus may do good work.

*Light source.* I have given up the electric arc because its light is far from uniform, especially with alternating current. The low voltage Mazda lamp is much more convenient, and gives a steady, even light. While the ribbon filament and the point-o-lit lamp are the best light sources for serious photomicrographic work, their high cost and short life is a disadvantage. They are necessary for the so-called critical illumination. The "Koehler Illumination" which will be described later, permits the use of non-uniform light sources. I have for several years used a 6 volt lamp of 50 watts capacity with concentrated filament such as is made for automobile search lights. It works very well with the "Koehler Illumination." The principle of this method is to form an image of the light source on the iris of the microscope and to project the field diaphragm which is mounted near the condenser of the lamp house, in the plane of the object.

In every case, we follow systematically a certain set of maneuvers. For example, we want a photomicrograph of a paraffin section stained with hematoxylin and eosin, in a magnification of  $\times 140$ . From the magnification table we learn that the objective 10 (N.A. 0.30) with eyepiece 7 and camera extension 50 cm. will be needed. The microscopic section is placed on the stage of the microscope and the microscope, without lenses or mirror, in horizontal position is inserted into the optical axis of the apparatus, between light source and camera. The ground glass of the camera has in the center a pencil cross to which a cover glass is fastened by means of canada balsam. The camera is in

MAGNIFICATION TABLE

MAGNIFICATION	OBJECTIVE	EYEPIECE	CAMERA EXTENSION
1 to 14 times	Tessar attached to camera	None	cm. Varies
15 times	3	5	25
30 times	3	5	50
35 times	5 (N.A. 0.15)	7	25
70 times	5	7	50
70 times	10 (N.A. 0.30)	7	25
140 times	10	7	50
140 times	20 (N.A. 0.65)	7	25
280 times	20	7	50
480 times	20	12	50
630 times	90 (N.A. 1.25)	7	25
1260 times	90	7	25
2160 times	90	12	50

Substage condenser: For objectives 10 and lower: use lower half of condenser (N.A. 0.4). For objectives 20 and higher: use the whole condenser (N.A. 1.4).

fixed position, while light source, condensers and microscope are movable. While looking at the ground glass of the camera, light source and microscope, without substage condenser, objective or eyepiece, are centered into the optical axis. When the field diaphragm and the substage iris are closed to a pinhole, the pencil cross of the ground glass must be seen in the center of a small light disc. The microscope is fixed in this position on the bench of the apparatus, the eyepiece is inserted and attached to the front board of the camera with a light-tight connector and the lower half of the substage condenser is inserted into the substage of the microscope. After screwing the objective into the nose-

piece of the microscope, the paraffin section is focused on the ground glass of the camera by turning the micrometer screw of the microscope.

Now the all important adjustment of the light is made according to "Koehler." (1) Aperture illumination: By moving the position of the condenser of the lamp house, an enlarged image of the light source is projected sharply on the iris of the microscope, so that it fills out the aperture of the diaphragm. (2) Field illumination: By moving the substage condenser, the margin of the closed field diaphragm which is in front of the light source condenser, is focused into the center of the ground glass of the camera.

These two steps are absolutely necessary to obtain homogeneous illumination of the microscopic field.

Then the filters are inserted into the holder in front of the lamp house. In

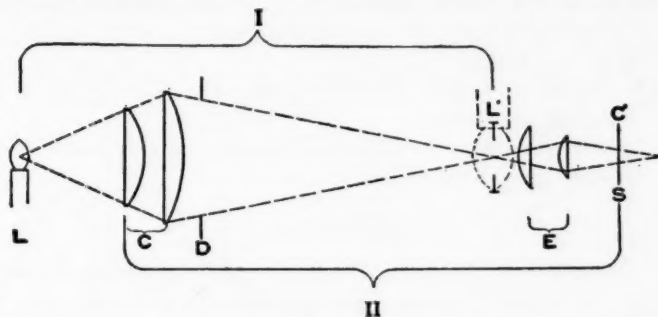


FIG. 3. DIAGRAM OF "KOEHLER ILLUMINATION" FROM BAUSCH AND LOMB CATALOGUE

(I) Aperture Illumination is accomplished by changing the position of the light source condenser C.

(II) Field Illumination is accomplished by changing the position of substage condenser E.

our case, with low magnification, we select Wratten filter B and G. The field diaphragm and the substage iris are opened to clear the microscopic field, not more than to  $2/3$ , otherwise haziness of the picture will result due to faulty reflections. Finally critical focusing is done with the micrometer screw of the microscope, using a special focusing magnifier on the clear center of the ground glass.

The ground glass of the camera is replaced by the plate holder and the exposure is made by removing a dark cardboard which is held between light source and microscope. This method is better than using the shutter of the camera, because it will avoid any vibration of the apparatus.

*Selection of objectives and eyepieces.* Before undertaking the photomicrography of any specimen, the lenses should be selected according to the desired magnification. Since the detail resolved in the microscopic picture depends entirely on the numerical aperture of the objective, the selection of the right

objective is most important. The general rule is that the highest acceptable magnification is 1000 times the numerical aperture of the objective in use.

From the table of magnification, it is evident that we prefer, for really crisp, sharply defined pictures, lens combinations where the magnification does not exceed 500 times the numerical aperture of the objective in use.

For magnifications lower than  $\times 15$ , tessar lenses which are fastened directly to the frontboard of the camera are used.

Photomicrographic technic looks complicated on paper, in reality it is a set of six simple maneuvers:

- (1) Insertion of the microscope into the optical axis of the apparatus, i.e. into a line which connects the center of the light source with the pencil cross of the ground glass of the camera.
- (2) Insertion of the right objective, eyepiece and substage condenser.
- (3) Adjustment of light (Koehler Illumination).
- (4) Insertion of filter.
- (5) Clearing of microscopic field by opening the field diaphragm and the substage condenser.
- (6) Critical focusing of the microscopic picture on the clear center of the ground glass of the camera.

#### CONCLUSIONS

Every large hospital should have a photographic department in charge of a biological photographer. In hospitals which cannot afford a fulltime photographer, the organization of a photographic department as division of the pathological laboratory is practical.

Standardization of the photographic procedures is necessary to obtain good results.

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## CHANCROIDAL VACCINE

### 1. A METHOD OF PREPARATION

### 2. ITS DIAGNOSTIC AND THERAPEUTIC VALUE\*

ROBERT B. GREENBLATT AND EVERETT S. SANDERSON

*From the University Clinic and the Departments of Pathology and Bacteriology,  
University of Georgia School of Medicine, Augusta, Georgia*

The great stimulus responsible for the present feverish activity in the problem of lymphopathia venerea is no doubt due to the widespread acceptance of the diagnostic test devised by Frei and to the isolation of the virus by Levaditi. As these cases are studied the difficulty in differentiating this disease from the very common affliction known as "chancroid" becomes increasingly evident. Just as in the history of the venereal diseases, the soft chancre in being the "vade mecum" of the hard chancre delayed their proper evaluation for so long a time because confirmatory laboratory evidence was lacking, so also the chancroidal bubo in being so closely simulated by the bubo of lymphogranuloma inguinale presents a similar problem.

For several years we have prepared an antigen from the pus aspirated from the buboes of lymphogranuloma inguinale according to the method of Frei. The importance of differentiating these buboes in certain moot cases is amply borne out by the following case.

*Problem Case 1.* A colored female age twenty-three, presented herself at the University clinic with a fluctuant bubo. A small primary sore had been present within the vagina for several weeks which, at examination, appeared healed. The blood Wassermann was negative; Frei test was strongly positive. No organisms could be demonstrated in the pus aspirated from the bubo either by direct smear or by routine cultures. Frei antigen was prepared and failed to elicit a positive response in other known positive cases. This curious fact soon

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unravelling itself when further investigation led us to believe that we had inadvertently prepared a chancroidal antigen from a patient with a positive Frei response. A positive reaction was invoked in numerous cases with typical chancroidal infection, in several of which bacilli resembling Ducrey could be demonstrated in smears from the primary lesion.

In December 1935, we were encouraged by the report of Cole and Levin<sup>1</sup> describing the intradermal reaction for chancroids with chancroidal bubo pus. We felt that our experiences substantiated their work. Credit is due them for drawing the attention of the American physician to this procedure. This method was first suggested by Frei and further investigated by Annuzzi<sup>2</sup> in Italy in 1928.

In our series of cases certain discrepancies between the intracutaneous chancroidal bubo pus test and the clinical and laboratory findings arose. In several cases in which the bacillus of Ducrey could be demonstrated either in the primary lesion or more rarely in pus of the bubo, a negative reaction was obtained with our chancroidal pus antigen. With a small supply of antigen obtained from Cole's clinic the same occasional discrepancy was noted. Bratzlavsky and Marenius<sup>3</sup> using pus antigen tested a series of cases and obtained a positive reaction in 81 per cent of the chancroidal positive cases. In our series of one hundred and sixty-eight intracutaneous chancroidal bubo pus tests we interpreted four false positive and thirty-two false negatives, thus obtaining 79 per cent correct reactions. We can affirm to the usefulness of this procedure and do not wish to detract from its merits. But the percentage of error, however small, does not permit of its general acceptance particularly when a vaccine may easily be prepared and found more reliable.

*Problem Case 2.* A white male aged forty-four, presented himself at the University clinic with an ulcer in the sulcus of the penis which had been present for three weeks. An indurated bubo was present in the right inguinal region. The blood Wassermann was negative; Frei test was strongly positive; intradermal chancroidal bubo pus test was negative. Using Teague and Deibert's technique the Ducrey bacillus was cultured from the ulcer. Repeated tests with our chancroidal pus antigen as well as that obtained from Cole's clinic remained negative as long as ten weeks afterwards.

This observation was noted in several other cases. The need for a more accurate diagnostic skin test was clearly pointed out by the above case. Upon further investigation positive reactions were repeatedly obtained with our vaccine which is described below. Dmelcos vaccine is used in Europe and in Canada both for diagnosis and therapy in chancroidal infections, and its use as a test is preferred to the use of sterilized pus. This is a standardized procedure whereas the use of pus is crude by comparison. As Eugene Traub<sup>4</sup> expressed it, "we shall eventually strive to get away from the use of human pus." However Dmelcos vaccine is not available since it cannot be obtained without a special permit in the United States. Cole in the discussion of his paper said: "It may be that some can grow the Ducrey bacillus with ease; unfortunately, that is not our experience in Cleveland. It is a most difficult procedure. We have been trying it for quite a few years." We have learned from other investigators elsewhere such as in New York and New Orleans, that this too is their experience. The chief obstacle lies in the difficulty in isolating the organism and the maintenance of its growth once it has been isolated. Several methods have been described for the cultivation of the organism which, in the experience of several investigators, have proven unsatisfactory.<sup>5</sup> We therefore set out to find a method for the cultivation of the bacillus which would lend itself with facility for the preparation of a vaccine; a method suited for the production of saline suspensions of the Ducrey bacillus and which would be generally acceptable because of its relative simplicity and accuracy.

Early in our work we were fortunate in isolating one strain by merely streaking the pus from a bubo directly on a blood agar plate. A colony was transferred to a tube of slanted infusion agar to the surface of which had been added approximately 1 cc. of human defibrinated blood. The upper portion of the tube was gently heated in a flame, the cotton plug pushed in a little and the tube then sealed with a tight fitting rubber stopper. This method of partial oxygen tension was used by Swartz<sup>6</sup> in cultivating the gonococcus, and has recently been advocated by Hunt<sup>7</sup> for the Ducrey bacillus. Our strains grew very well under these

conditions, especially in the blood itself. We have observed that this and subsequent strains of the organism grow very poorly as surface growth, even when the blood is incorporated in the agar before solidification.

#### TECHNIQUE FOR THE CULTIVATION OF THE DUCREY BACILLUS

In our search for a medium which would adequately support the growth of Ducrey's bacillus, the following method appeared to answer our purpose:

1. In 8 cc. aluminum screw top ampules. Put up in 4 cc. amounts a 0.5 per cent soft agar with a veal infusion base.
2. When culturing, added 1 cc. of human defibrinated blood.
3. As inoculum use 0.2 cc. of pus.
4. Incubate at 37°C. for two to four days.

The longevity of such cultures has not been determined but successful transplants after one week have been obtained when kept at room temperature following a two day incubation at 37°C. Because the organism grew so well its recovery from buboes was then attempted by the use of this method. In quick succession three additional strains were obtained, two in pure culture and one mixed with a staphylococcus. This procedure eliminates the use of clotted rabbit blood advocated by Teague and Deibert<sup>8</sup> as a preliminary culture medium.

#### PREPARATION OF VACCINE

Hunt used washed saline suspensions of Ducrey bacilli from surface growth on blood agar. As mentioned above our strains grew very sparsely on the surface, an observation made by Hunt<sup>9</sup> on one we sent him. The growth however, was very luxuriant in the blood when placed directly on the surface of an infusion agar slant.

#### TECHNIQUE FOR THE PREPARATION OF THE VACCINE

In the preparation of the vaccine the stock culture is transferred to the blood on the surface of a veal infusion agar slant. After two or three days incubation at 37°C. the fluid blood is removed and added to approximately 25 cc. of distilled water and centrifugated at high speed for thirty minutes. The supernatant fluid is removed and 25 cc. of distilled water again added and centrifugation repeated. Two such washings will usually remove most of the haemoglobin and serum. The sediment composed of bacteria and cellular

debris is suspended in 10 cc. of sterile salt solution and heated for one hour in a water bath at 60°C. Merthiolate 1:10,000 is added as a preservative.

#### INTERPRETATION OF THE SKIN TEST

Using 0.1 cc. of the vaccine as an intracutaneous test on the forearm a very marked skin reaction resulted in cases with a known chancroid infection. These have been repeatedly in agreement with the vaccine obtained from Hunt. The reactions were read at the end of forty-eight to seventy-two hours and graded from one to four plus and were usually clear-cut. These were stronger and more persistent than with chancroidal pus antigen. In the greater number of cases a positive reaction may be expected two to five weeks after infection. In one hundred sixty-four tests that were made with our vaccine six false positives and four false negatives occurred, a percentage of 94 correct reactions. The same dosage was used at weekly intervals as a therapeutic measure thereby providing a double check on our tests, for these too were read at the end of seventy-two hours. Actually the correct diagnosis was made with greater frequency; for instance, a patient may receive four vaccine tests on different occasions and respond only to three. The one negative test was interpreted as false.

#### THERAPEUTIC VALUE

It is difficult to draw conclusions in the treatment of a disease that is generally recognized to be self limited. However, in most of the old rebellious chronic chancroids as well as the fresh cases, the subjective and objective improvement was so striking that these patients were profuse in expressing their gratitude. We conscientiously feel that the improvement so manifested was not psychic, but real. The course of the disease was shortened, the buboes subsided more frequently and earlier. The improvement in the well-being of the patient after one or two intracutaneous tests was almost dramatic.

#### COMMENT AND CONCLUSIONS

Fifty-four cases of chancroidal infection were carefully studied and conclusions drawn from one hundred and sixty-four chan-

chroidal vaccine reactions and one hundred and sixty-eight chancroidal bubo pus antigen tests. That a suspension of culture is superior to a preparation made directly from bubo pus is amply borne out by these diagnostic procedures. Seventeen of these patients also gave a positive Frei reaction. It was evident that every case presenting a bubo should be tested with chancroidal vaccine as well as Frei antigen.

#### SUMMARY

A facile method for the cultivation of the Ducrey bacillus and the preparation of a successful chancroidal vaccine is outlined for diagnostic and therapeutic use.

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A. HELWIG, M.D.

O. SAPHIR, M.D.

M. WARWICK, M.D.

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## THE HOSPITAL AND THE NECROPSY

## 1931

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## NECROPSY FINDINGS

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## OBTAINING PERMISSION FOR NECROPSY

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- \*44. Injuries to physician or his assistants in course of medico-legal necropsies. Renoux. *Ann. de Méd. Lég.*, **14**: 407-408, 1934.

## VALUE OF NECROPSY FOR TEACHING PURPOSES

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2. Autopsies in Relation to Teaching. W. A. Bloedern. *Jour. Am. Med. Coll.*, **6**: 193, 1931.
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## THE NECROPSY AND FAMOUS PEOPLE

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HISTORY OF NECROPSIES

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MISCELLANEOUS

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(To be continued)

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\* Article abstracted, see Part II.

## NEWS AND NOTICES

Under the direction of Jack C. Norris, M.D., Chairman of the Publicity Committee, arrangements have been made to cover the Philadelphia convention in a satisfactory and capable manner.

Publicity Headquarters will be in the Bellevue Stratford Hotel and direct contact has been secured with five of the largest Philadelphia newspapers and also with the Associated Press. All arrangements for newspaper articles and statements and for broadcasts also, will be decided upon, arranged for, and announced from the Publicity Headquarters.

Under this arrangement all newspaper articles will be controlled in such a manner as to assure that they will be authentic, free from undesirable sensationalism and of a character in keeping with the prestige of the Society.

In order that the Publicity Headquarters may be fully prepared to handle this important matter Dr. Norris urgently requests those who will present papers at this meeting to send him as soon as possible a copy of the paper from which extracts suitable for publication may be prepared for distribution to the press.

By this means sensationalism can be avoided and accurate news reports assured.

*All those presenting papers will therefore please send a copy of the paper to Jack C. Norris, M.D., Suite 810, Doctors Building, Atlanta, Georgia.*

The success or failure of the publicity arrangements depends upon *your* coöperation.

Word has been received of the death of E. E. Laubaugh, M.D., of Boise, Idaho, on December 13th, 1936 of pneumonia.

### Committee Appointments:

Wm. Jones Deadman, M.D.: Local Counsellor for Canada.

David R. Meranze, M.D., Philadelphia: Committee on Commercial Exhibits.

E. L. Thoringer, M.D., Milwaukee: Committee on Life Membership.

DR. EMSLEY T. JOHNSON

Dr. Johnson, while only a member of this Society for a short while, had stamped his friendly personality on the officers and numerous members during the Society's annual meeting in Kansas City in 1936.

Dr. Johnson was born January 7, 1892; received his A.B. and M.D. at the University of Kansas. After several years of hospital work and one year of instructorship in Pathology, University of Kansas, he became assistant pathologist at Research Hospital, Kansas City, in 1925. While here, his friendly jovial spirit, his inquisitiveness as to the cause of death, and his ability to interest others in his post mortem findings became known to the general profession of his city.

In 1931 he accepted the position of pathologist to St. Joseph Hospital, Kansas City, which position he held until his sudden death while attending his professional duties on November 20, 1936, from cerebral hemorrhage.

Under his guidance, the laboratory service at his hospital was second to none in his city. The autopsy rating rose rapidly so that in 1934 his hospital had the first place in the rating of autopsy percentage of the American Medical Association. His ability and popularity is attested by the fact that within ten years of his arrival in his city he was elected President of Jackson County Medical Society.

"He met death in the prime of life, at the height of a brilliant career; he met it bravely, without whimpering; he leaves to his family and friends a heritage rich in those virtues most prized by men."

—F. C. NARR.



## BOOK REVIEWS

*Textbook of Pathology.* By SIR ROBERT MUIR, Professor of Pathology, University of Glasgow. Cloth, Ed. 4, 994 pp.; 571 figures, \$10.00. William Wood & Co., Baltimore, Md.

That this useful book has reached a fourth edition will not be surprising to those familiar with it who must, by now, be legion.

Intended primarily as a textbook for the students in Glasgow University, in the twelve years elapsing since its first appearance it has taken its place as a reference text of value, not only to students, but to physicians and pathologists as well.

As heretofore, the new edition embodies many changes throughout the text which is thus brought in line with the advances of the last three years. A number of new illustrations have also been added.

This book may be recommended as a useful and valuable text.

*Amebiasis and Amebic Dysentery.* By CHARLES T. CRAIG, M.D., Colonel, U. S. Army, Retired. Cloth, pp. 315, 54 figures, \$5.00. Charles C. Thomas, Springfield, Ill.

The name of the author on the title page of this book is ipso facto evidence of the authoritative character of the text.

The outbreak of amebiasis in Chicago in 1933 focussed attention upon this disease and again emphasized its lack of geographical limitations.

That amebiasis is a disease of increasing importance the studies and reports of recent years have made more and more apparent. That Colonel Craig would present an eminently practical and usable text bearing the stamp of authority and experience could be expected from his extensive experience in this and allied fields. The reader may approach this book with full confidence that his expectations will not be disappointed.

The format is excellent and the numerous illustrations of high quality.

This book may be recommended without reserve.

*Histological Technic.* By ARAM A. KRAJIAN, Department of Pathology, Los Angeles County General Hospital. Privately printed, cloth pp. 217.

In this book the author presents the histological methods used in the laboratories of the Los Angeles County General Hospital.

The technic is given in full detail and in many instances embodies modifications originated by the author.

A detailed index facilitates finding any particular method desired.

As a reference text the volume should prove useful to laboratory workers.

*Body Water: The Exchange of Fluids in Man.* By JOHN P. PETERS, M.D., Professor of Internal Medicine, Yale University School of Medicine. Cloth, 405 pp., 4 figures, \$4.00. Chas. C. Thomas, Springfield, Ill.

As stated by the author in his Preface, this book is not intended as an unprejudiced review of the subject with which it deals, but is the defense of a thesis based upon extensive investigations by Dr. Peters and his associates upon the distribution and movement of solutes and water in the human body.

Arising from a desire to elucidate the phenomena seen in nephritis, the studies of this group have comprised investigations in diverse fields and the results obtained have added in no small measure to the understanding of the phenomena involved in the fluid exchange of the human body. The data presented in this volume, therefore, are of great interest and importance to all who are interested in the phenomena of disease in general and in those accompanying nephritis in particular. It is in relation to this latter problem that this book will arouse the greatest interest and may be consulted with the greatest profit.

The comprehensive character of the investigations is indicated by the chapter headings: Chemical forces which control exchanges of fluid and solutes; The nature and movements of interstitial fluid and lymph; Exchanges between blood and interstitial fluids; Serous fluids and transudates; Exchanges between tissue cells and interstitial fluids; Water of oxidation and the

losses through skin and respiratory passages; Alimentary exchanges; The general nature of renal activity; Renal excretion of filtrable organic solutes; Renal excretion of water and inorganic salts; and Nervous and hormonal control of urine excretion.

The book abounds in succinct and almost epigrammatic summations. For example: "Consideration of the nature of renal function, especially in pathological conditions, has been too often prejudiced by the fact that the kidneys are excretory organs. When regarded unconditionally in this light their responses frequently appear utterly stupid, in violation of the dictates of biological automatism" (p. 203).

"In clinical medicine undue stress upon excretory function has led to endless search for the cause of 'uremia' in 'retention products' to the almost complete neglect of the disorganization of body media which results from the failure of diseased kidneys to prevent escape from the body of water and solutes" (p. 204).

"The so-called critical level of protein at which edema appears is only the level at which the accumulation of fluid becomes grossly evident, because it is great enough to form pools under the influence of gravity" (p. 49).

It is impossible to read this book without interest and still less possible to lay it down without having profited by its study.

The style is clear and easy to read; the type and format acceptable. There is an excellent index and bibliography. The book should appeal to physiologist, pathologist, and clinician alike.

*The Lung.* By WILLIAM SNOW MILLER, Emeritus Professor of Anatomy, University of Wisconsin. Cloth, 209 pp.; 152 figures; 18 colored plates, \$7.50. Charles C. Thomas, Springfield, Ill.

This is probably the most complete monograph yet published upon the structure of the lung and represents the summation of the author's studies over a period of forty-seven years.

Dr. Miller's interest in this subject was first awakened by the suggestion, first made in 1887 by Professor Delafield, that free

communications existed between the air spaces in the lungs. From that time on he has continued the studies the results of which are so clearly and comprehensively presented, and so beautifully illustrated in this book.

The book consists of twelve chapters in which are discussed: The Lungs; The Trachea and Bronchi; The Air Spaces; The Blood Vessels; The Lymphatics; The Pulmonary Lymphoid Tissue; The Nerves; The Pleura; Key Points, The Acinus. An additional chapter presents a complete historical sketch of the literature on the lungs in which, in chronological order, the work of various investigators from Malpighi (1628) to Braus (1924) is summarized.

Dr. Miller is to be congratulated upon a notable contribution and the publisher commended upon a presentation worthy of the work.

This volume should have a wide and varied appeal. Not only the anatomist, the physiologist, and the pathologist, but also the teacher of surgery and the chest surgeon may turn to it with confidence as a reliable source of pertinent information. Those interested in tuberculosis should find this book of great interest.

The volume is beautifully printed and excellently and profusely illustrated.

There is a complete bibliography and index.

## EDITORIALS

### THE CLINICAL LABORATORY AND THE PUBLIC HEALTH

During the past twenty years national and state health departments have made strenuous efforts, in the interest of public health, to establish laboratory facilities for the investigation and control of certain infectious and contagious diseases. At first these laboratory activities were confined to the investigation of problems dealing with the etiology and epidemiology of epidemic and contagious diseases. The splendid work of the National Institute of Health of the Public Health Service in Washington is of course well known and represents the ideal sphere of operation of governmental agencies. The state health departments on the other hand, have assumed from time to time, new and additional responsibilities. Activities of state and municipal laboratories under their direction have been extended far beyond the fields of epidemiology and quarantine in such diseases as typhoid, dysentery, malaria, diptheria, etc, to include a private diagnostic service for the individual. In some public health laboratories, so called, individual blood counts, urine analyses and nitrogen determinations can be obtained, and in some instances advice and counsel regarding the individual case have been given. In other words, some of our public health laboratories are entering into the practice of medicine.

Few would be willing to question the good intentions that have motivated those responsible for this invasion by governmental agencies. It is admitted that the basis for the extension of this service was to make available to physicians and patients many clinical laboratory examinations not obtainable in small communities. This individual service was first limited to the examination of throat cultures for diptheria, cultures and agglutination tests for typhoid fever, and later included Wasserman tests. As time elapsed this service has been extended in many states to the entire field of Clinical Pathology.

At the time that health departments adopted this policy, the field of clinical pathology was rapidly expanding to supply the increasing demands made by the progressive practitioner of medicine. However, as the state and later municipal health departments expanded and encouraged physicians to use their facilities without cost to them, but not necessarily without cost to the patient, the inevitable result occurred; namely, there has been a noticeable tendency to abandon the field of clinical pathology for more promising specialties of medicine. As a consequence, comparatively few able men have specialized in clinical pathology within the past ten to fifteen years. On the other hand, the need for such services, particularly outside of the Cosmopolitan areas, has grown tremendously. The question to be answered is this: have the activities of public health laboratories been beneficial to public health in the long run? The answer to this question by one in close contact with this problem is emphatically "No."

Public health measures, strictly speaking, are concerned with the investigation and control of epidemic diseases, the control of water, milk and food supplies, and the promulgation and enforcement of quarantine measures. But there is a type of semi-public health service—that is, where smaller aggregates or groups of the public are concerned—requiring a different medical approach and set-up. It centers about and radiates from the hospital, whether it be a municipal, religious, or private institution. This service is primarily concerned with individualized problems of disease. The spearhead of the attack is the clinical pathologic conference, that is, the group-study of disease as manifested in the individual. Frequently it falls to the lot of the clinical pathologist to plan for and lead these conferences. Usually the hospital staff, as a whole, is coöperative, the hospital administration is interested, The American Society for the Control of Cancer has been a warm advocate, The American College of Surgeons, and The American College of Physicians have taken it up. The Council on Medical Education of the American Medical Association has all but made it mandatory for the approval of internships. No one doubts its value—but



where are the key men, the clinical pathologists, going to come from in the future—if the field of clinical pathology is to be economically ruined by the state public health laboratory? Some of us who are close to these problems wonder, at times, whether our clinical brethren have not been rather short sighted in encouraging the state laboratory movement, especially when they see their patients leaving through lack of local diagnostic facilities to seek better equipped medical centers.

Is it not time, then, to take an inventory of present trends and attack our problems, for it concerns the entire profession, in the light of existing conditions? What is the solution? The answer is plain—encourage local communities to develop and support local laboratories conducted independently by well-trained clinical pathologists. This has been accomplished in many localities by the concerted action of local physicians, coöperating with local city and county health officers and in many instances with local hospitals. Such coöperative movement has created a sufficient volume of local work to maintain a well-equipped and well-manned laboratory directed by a clinical pathologist who is always available for consultation regarding the need for, and the proper interpretation of laboratory aids for diagnosis and treatment. Thus, such a community becomes independent of the distant state laboratory for it obtains prompt service in the examination of throat cultures, blood films, serologic and tissue specimens, to say nothing of the important examinations which must be made on the spot.

This program does not imply that the present State Health departments should be closed. Indeed they should expand their activities, but, in the fields of quarantine research and standardization of independent laboratories. Through such activities the efficiency of coöperating hospital and private laboratories could be raised and inefficient ones eliminated.

Great strides in the solution of these problems could be made by active coöperation between public health departments and The American Society of Clinical Pathologists. A very practical example of this coöperation was seen in the recently conducted evaluation of sero-diagnostic methods of syphilis. It has spon-

sored the clinical pathological conference and conducts each year a tumor seminar for its members. Recently, the society inaugurated steps leading to the formation of The American Board of Pathology, demonstrating in this practical way, its desire to conform—nay, lead—in the best traditions of Medicine. Any steps which would discourage the growth of the practice of Clinical Pathology in local communities would inevitably defeat the purposes for which the medical profession exists; namely, the protection and welfare of the patient.

—A. S. GIORDANO.

#### THE TECHNICAL SUPPLEMENT

It is, perhaps, a truism to say that, as is, indeed, true of advances in medicine in general, the laboratory technic of tomorrow is to be found in the medical literature of today. It is equally true that many of these newer methods do not reach the text books and laboratory manuals until long after their original publication, sometimes because they await the test of trial and experience, sometimes because they fail to receive the recognition and dissemination which is properly their due.

The American Society of Clinical Pathologists furnishes a natural forum for the presentation of such material and a natural jury by which its worth may be estimated, for how shall the value of a laboratory procedure be known until it has been passed upon by the clinical pathologists in whose field it falls?

As an aid to the dissemination of such material, and to bring to the attention of our members and subscribers not only newer methods and modifications the value of which has been proved, but also methods which on *prima facie* evidence seem worthy of trial in order that their real worth may be demonstrated, the Journal will shortly inaugurate a new department to be known as the Technical Supplement.

As the name implies, this section of from 15–20 pages per issue will be devoted to technical procedures. Not to those with which the competent laboratory worker is already familiar, but to those which seem worthy of trial or which the experience of others has found to be good.

This material will be drawn from several sources: from original contributions embodying the work and experience of the members of this society; from original contributions upon special subjects by those in a position to discuss them with authority; and from the current literature in which appear many methods of interest to the laboratory worker but not always brought to his attention.

The technical section will be not only incorporated in the Journal proper but will also be inserted as a reprint in each issue. These reprints, punched to fit the standard binders, may be used in the laboratory as a working text and, if desired, may be secured in sufficient quantity to supply the laboratory personnel with an individual copy.

Furthermore, at the end of each year the technical material published will be available at a nominal price as a separate Technical Supplement to the Journal.

The inauguration of this section is made possible by the support and coöperation of the Board of Registry by whom, at the start, it will be underwritten. Its continuance will depend upon the support and coöperation extended by the Society at large.

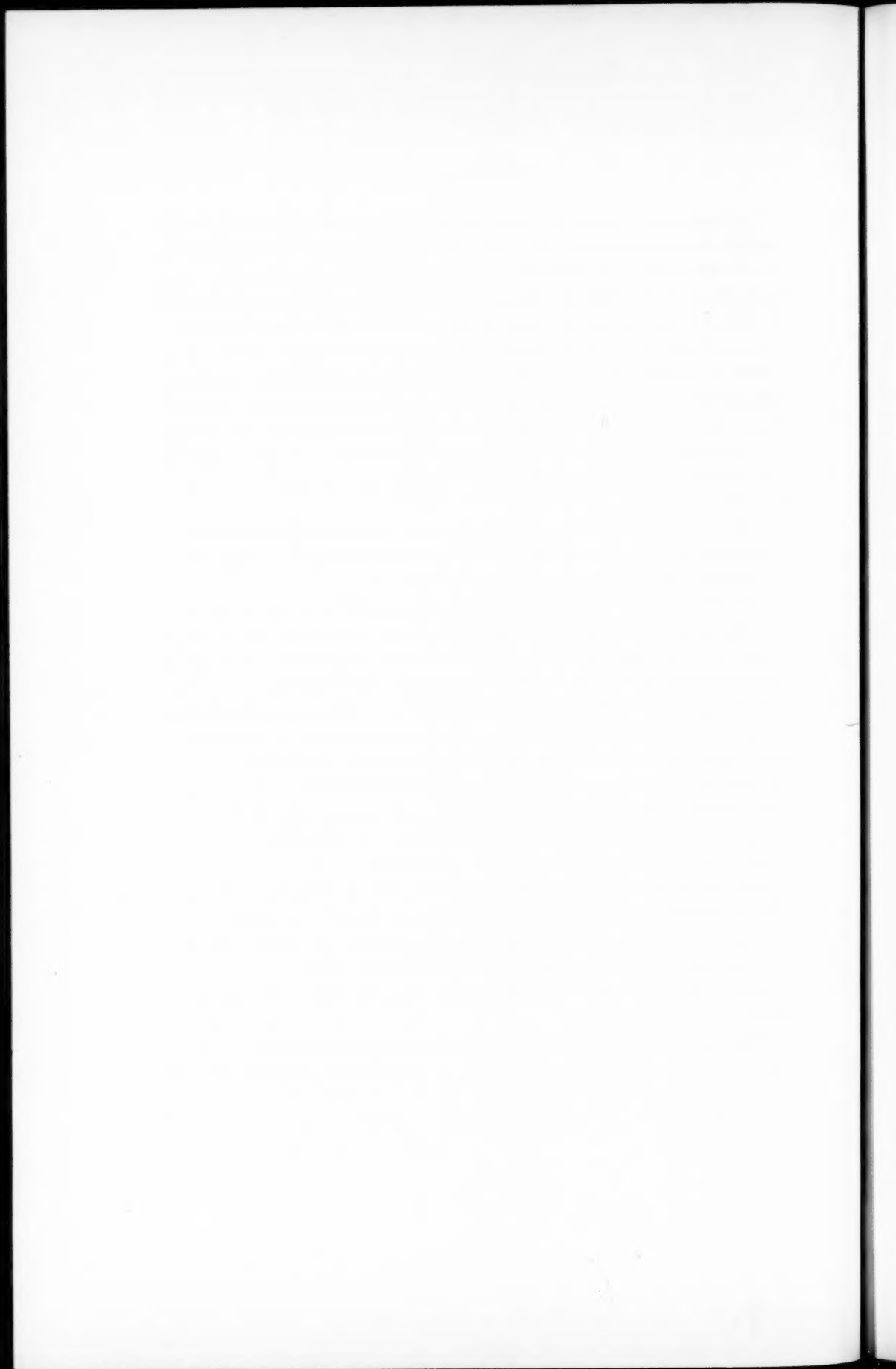
It is expected and hoped that all who have developed in their laboratory modifications leading to improvements in accepted laboratory procedures; who have devised new procedures, new apparatus, or useful laboratory aids or "short-cuts"; or who in their reading discover methods worthy of wider trial, will offer them for publication in this department. Full credit will be given in every instance.

The Sub-Editor for this section will be W. S. Thomas, M.D., Clifton Springs Sanitarium, Clifton Springs, New York, who, with the help of assistants, will select and prepare the material for the technical section in collaboration with the Editor.

All material for this section should be sent either to Doctor Thomas or to the Editor.

May we count upon YOUR coöperation and assistance?

—R. A. KILDUFFE.



## THE PREPARATION OF DEXTROSE SOLUTION FOR INTRAVENOUS ADMINISTRATION\*

WILLIAM J. ELSEER AND RALPH G. STILLMAN

*From the Central Laboratories, New York Hospital and the Department of Applied Pathology and Bacteriology, Cornell University Medical College*

The feasibility of intravenous medication was demonstrated by Christopher Wren in 1656 and Purman in 1692 described the unpleasant effects of paravenous infiltration with injection fluid.<sup>12,36</sup> However, it was not until Wood introduced the use of the Pravaz syringe for subcutaneous injection in 1853 and Land-erer in Leipzig carried out infusion of salt solution into the vein in 1881 that intravenous injection was generally adopted.<sup>36</sup> It is important to note that before 1908 it is difficult to find reports of reactions following this operation. At that time the water used was not always freshly distilled, sometimes not distilled at all, and little attention was paid to the rate of administration. After the introduction of salvarsan there began to appear accounts of patients who had chills and fever following its administration. At that time Wechsellmann<sup>45</sup> pointed out that the distilled water used was likely to be old and contaminated with bacteria. He suggested that immediately after distillation the water to be used should be stored under "strictly aseptic conditions" and this precaution reduced the number of reactions. Muller<sup>30</sup> showed that the commercial distilled water of that time could contain living and dead bacteria in quantities of from 20,000 to 6,050,000 per cubic centimeter and it was generally believed that the reactions that occurred were due to the effect of foreign proteins.

During the World War intravenous therapeutics was applied more frequently. The discovery of the blood groups had made

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blood transfusion comparatively safe and solutions other than that of sodium chloride were also used. "Gum-glucose" was used extensively in the British army but in our hospitals its injection was followed by a number of reactions and its use was abandoned. The cause of these reactions was not determined but it was known that the solution was transported at times under unfavorable conditions and subjected to extremes of temperature. After the War, methods for the preparation of a gum-glucose solution safe for intravenous injection were developed in several places including the New York Hospital.<sup>26</sup>

With increased use of glucose there appeared also an increased number of reactions and studies undertaken in the attempt to discover the reasons for their occurrence, revealed the following factors which deserve consideration in this connection:

- I. Materials
  - 1. Water
  - 2. Dextrose
  - 3. Sodium chloride
  - 4. Containers
- II. The solution
  - 1. Mode of preparation of solution
  - 2. Clarification
  - 3. Sterilization
  - 4. Characteristics of the solution
  - 5. Storage
- III. Equipment for administration
  - 1. Glassware
  - 2. Rubber tubing
- IV. Administration of the solution
  - 1. Temperature
  - 2. Rate of flow
  - 3. Phleboclysis
  - 4. Skill of operator
- V. Susceptibility of the patient

Our interest in the subject began in 1920 with the development of a method for the preparation of dextrose and acacia ("gum-glucose") solution.<sup>26</sup> In 1925 we began a study of dextrose solution and developed the method of preparation now in use in the New York Hospital. Up to January 1, 1936 we have issued more



than 33,000 100 cc. flasks of 50 per cent dextrose of which about 90 per cent were injected, either as such or diluted, directly into the circulation. The remainder was administered by hypodermoclysis.

Discussion of possible factors in causation of reactions.

#### MATERIALS

*Water.* Shortly before this time, there appeared Seibert's important contributions.<sup>37, 38, 39</sup> She concluded that freshly and properly distilled water was always non-pyrogenic when tested within 24 hours after distillation and that it would remain so if sterilized within this period. She isolated from old distilled water an organism which she believed to be responsible for reactions and from her work concluded that such reactions were not produced by rapidity of injection, pH of the solution, occurrence of hemolysis or specific ion effect. Seibert also pointed out the necessity for proper design of distilling apparatus so that spray cannot be carried over into the distillate.

Our attempts to grow this organism, even from very old distilled water, were not successful and the cultures which Seibert sent us for inoculation studies, failed to grow in subcultures. Banks claims<sup>1</sup> all waters showing pyrogen contain *Pseudomonas acissa* or *Pseudomonas ureae*. The reactions that occur are similar to those following the injection of foreign protein so it seems probable that the growth in a water of any one of several organisms may result in its acquiring pyrogenic properties.

At about the same time there arose a vogue for doubly or triply distilled water. We have<sup>6</sup> set forth our criticism of the belief that such water has superior properties and have shown that a single distillation, properly carried out in a correctly designed apparatus will remove all pyrogenic substance, at least when dealing with New York City water which is relatively pure. We use a steam-operated Barnstead still provided with proper baffles, which is cleaned with such frequency that there is little tendency for the boiling water to foam.

This water when freshly distilled is safe but may become contaminated by improper handling. The reservoir in which the

water is collected should be made of glass and used for no other purpose. Under no circumstances should water be allowed to stand in it for more than 24 hours and the entrance of dust should be prevented as far as possible. We have used an ordinary 5 gallon glass demijohn, thoroughly rinsed with freshly distilled water before use and when not in use kept inverted on a tripod. This insures thorough drainage and the small amount of water that remains in the interior evaporates before growth of contaminating organisms becomes likely. We have not used Carter's test<sup>20,2</sup> for pyrogenic substance. It is a sensitive test for organic matter but it seems unlikely that a negative result means with certainty the absence of sufficient such matter to produce a reaction. Banks<sup>1</sup> says it is wholly inadequate.

Freshly distilled water must be used in preparation of the solution *within 24 hours* after distillation. We always use the water on the same day but it is believed the 24-hour period is safe. For most solutions sterilization also must be carried out within 24 hours but with concentrated dextrose solutions, because of their bactericidal properties, sterilization may be safely deferred until the following day. Only molds and yeasts will grow in them and these develop slowly.

*Dextrose.* Darrow<sup>4</sup> blames "bulk glucose" for some of his reactions and Fuqua<sup>11</sup> says the U. S. P. article should not be used. Thompson<sup>46</sup> recommends U. S. P. dextrose, while the majority of authors advise the grade known as "C. P. Anhydrous." We first used "Difco" anhydrous dextrose but the cost was excessive and we changed to Merck's "Dextrose, C. P. Anhydrous" and later also used Squibb's Anhydrous Dextrose. We tried one cheaper brand said to be of U. S. P. grade with which we produced a clinically satisfactory solution but difficulties in filtration and the presence of some colloidal material led us to abandon it. U. S. P. Dextrose contains not more than 10 per cent water, which must be borne in mind when calculating the amount of material needed for the solution. Other contaminants are dextrin, chlorides and sulphates, none of which is likely to be of importance in the production of reactions. The chief difference among the several brands, or even among different lots of the same brand is the ease

with which they go into solution, said to be due to differences in the size of the dextrose particle. Deviations in the color of the solution from colorless to a pale yellow appear to be without significance. Ordinarily little information can be obtained from the manufacturer and the only really satisfactory test is the use of a product in fairly large quantities and observation of its effect upon patients. If it passes this test and is free from actually poisonous ingredients such as arsenic it may be regarded as suitable.

*Sodium chloride.* Since dextrose is frequently administered in normal salt solution it is desirable to say a word about the preparation of the latter. U. S. P. sodium chloride or any similar or better grade may be purchased in the form of small crystals or as 9 gram tablets. Nine grams of salt are placed in a clean 1 liter Pyrex boiling flask which is then filled with freshly distilled water. The mouth of the flask is closed with a cellophane cap and the solution sterilized in the autoclave for 30 minutes at a pressure of 15 pounds. In our experience filtration is not necessary. This solution is used as desired for intravenous administration, either by itself or as a diluent for 50 per cent dextrose. In 8 years use of this salt solution we have seen but one reaction which is said to have followed the injection of a cool solution.

*Containers.* We have used only Pyrex flasks for the finished solution. Pyrex glass is highly resistant to both mechanical and chemical attack and its use is therefore economical though the first cost is high. Resistance glass has been used, especially for ampules but the possibility of using soft glass has not been sufficiently investigated. Whitney<sup>46</sup> uses bottles of a "harder than soft" glass and Friedmann<sup>9</sup> used ordinary wine bottles during the war. When dealing with solutions of dextrose, the use of soft glass has certain theoretical advantages for alkali given off would tend to neutralize the acidity that develops on standing. On the other hand, caramelization proceeds more rapidly in solutions that have been neutralized and this may be regarded as a disadvantage. The question can be answered only by experimentation and the use upon patients of reasonably large quantities of solutions so stored. Soft glass is fragile and will, therefore, be expensive if flasks are used.

## THE SOLUTION

*Mode of preparation.* Dextrose tends to cake when dropped into water and the process of solution is accompanied by absorption of heat. In order to hasten the procedure the water is warmed and the dextrose added slowly with constant stirring. For the preparation of 6 liters of solution, it is convenient to use a 2 gallon wide-mouth bottle and stir with a glass rod. When large quantities are being made it is advantageous to make up lots of 24 liters in white enamelware stock-pots which should be used for no other purpose. This size was selected as the largest that could be handled readily. Since dextrose adds bulk to the solution, when making 24 liters, the sugar is dissolved in not more than 18 liters of freshly distilled water and water is added up to volume after solution is complete. As mentioned above, different brands and even different lots of the same brand may show differences in the time needed for complete solution, but for the brands we have used the time required is not more than a few minutes.

*Clarification.* Clarification of the solution is necessary and by most authors is accomplished by filtration through paper. Cheap papers may contain substances that are dissolved out by the solution and those of loose texture may shed fibers so that a good grade of paper or even hardened paper is usually recommended. The use of paper is likely to be expensive and to prolong the time of preparation. In our method clarification and sterilization are combined in one procedure.

The relation of the presence of particles to the occurrence of reactions is a subject that has not been generally discussed. Banks<sup>1</sup> says "particulate matter in dense concentration" has no effect while Darrow<sup>4</sup> thinks that particles of gauze, cotton or glass may be responsible for chills and Falk<sup>7</sup> advises filtration "at least three times" through the best paper. Heubner (cit. by Friedmann<sup>9</sup>) was able to produce fever by the intravenous injection of finely divided particles of paraffin. Such reactions are believed to be analogous to those occurring after the transfusion of incompatible blood. The chill appears within a few minutes of the introduction of the blood and is thought to be due to the lodgment of minute emboli of agglutinated cells. Kyes and Carey<sup>21</sup>

think the introduction of particulate matter into the circulation incites the formation of fibrin emboli. Little<sup>25</sup> agrees and Kyes and Strauser<sup>22</sup> state that the administration of heparin which inhibits fibrin formation, will prevent such reactions.

We have seen one reaction apparently due to particulate matter. Our "gum-glucose" was prepared by adding calcium carbonate before sterilization. On one occasion intravenous injection of the contents of a bottle of this solution used inadvertently only a short time after its preparation when it still contained particles of calcium carbonate in suspension was followed by a chill. It is generally agreed that solutions for intravenous use should be water-clear.

*Sterilization.* Sterilization of glucose solutions seems always to be accomplished by heat. Some of the methods recommended appear to be based upon a misconception of the manner in which heat may be applied effectively in killing bacteria and in all of them the changes which take place in dextrose exposed to heat have failed to receive sufficient consideration.

Grulee and Sanford<sup>13</sup> and Sanford and Heitmeyer<sup>24</sup> sterilize dextrose in the dry state, protecting it against moisture with a layer of calcium chloride and autoclaving it for 20 minutes and claim that shock is thus insured against. Shohl and Beal<sup>40</sup> use a similar method but autoclave only 2 minutes at 15 pounds, while Schwentker<sup>35</sup> seals the dry powder in a tube and heats it on a boiling water bath for 30 minutes on each of 3 successive days! The powder is then dissolved in sterile water, filtered or not filtered and is used at once. These authors apparently do not realize that their methods consist of the application of dry heat, the least efficient method of sterilization by heat and that the vegetative forms of bacteria and to a greater degree the spores require a higher temperature for their destruction when dry heat is used than when they are exposed to some form of moist heat. The success of the methods is probably due to the excellent quality of the dextrose used and to administration promptly after preparation.

Fractional sterilization is especially unsuited to dry or concentrated solutions of dextrose. It is based upon the principle that



moist heat at 100°C. for 15 to 20 minutes is sufficient to kill the vegetative forms of bacteria and that spores will develop into vegetative forms in the intervals if the medium is suitable for growth. We shall show that 50 per cent dextrose is not a suitable medium for bacterial growth and spores in it will not develop into vegetative forms.

The majority of authors recommend autoclaving at 5 to 15 pounds pressure for 15 to 40 minutes and we are informed that in some laboratories a temperature of 80°C. for 45 minutes is used. It has long been known that when a solution of dextrose stands at room temperature it gradually develops acidity due to breaking down of dextrose. Williams and Swett<sup>47</sup> found that 10 per cent dextrose of pH 6.20 when fresh, changed to pH 5.5 in

TABLE 1  
GUM-GLUCOSE (20 PER CENT DEXTROSE; 6 PER CENT ACACIA)

	pH
Original solution.....	4.7
Neutralized with NaHCO <sub>3</sub> .....	8.3
Autoclaved 30 minutes at 15 pounds.....	6.1
Neutralized with NaHCO <sub>3</sub> , filtered.....	8.4
Autoclaved 30 minutes at 15 pounds.....	5.8

24 hours and to pH 4.15 in 48 hours; when boiled 10 minutes, the pH dropped to 5.47; when a similar solution with pH 7.01 when fresh was autoclaved the pH dropped to 4.63. Our studies show it is not possible to prevent this change by neutralization with sodium bicarbonate and that the greater the alkalinity when heat is applied, the more profound is the change in the solution (table 1).

We prevented this change in pH upon autoclaving by adopting the well-known method of adding powdered calcium carbonate to the solution before heating. When thus treated 50 per cent dextrose after autoclaving 30 minutes at 15 pounds showed a pH of from 6.7 to 6.8. It also showed a certain amount of caramelization so that some destruction of dextrose had occurred though the pH remained unchanged. We had practically no reactions



with this solution but the method required not only standing for sedimentation of the calcium carbonate but also decanting of the clear supernatant fluid into sterile flasks for use, so that it was cumbersome and wasteful. The change in color due to caramelization was not accompanied by unfavorable results.

Since exposure of solutions of dextrose to heat results in more or less change in the sugar, we believed it was not necessary to show that this change was actually deleterious and concluded that the use of heat must be avoided in producing a perfect solution. We recognized that filtration through a bacteria-removing filter is the method of sterilization that will cause the least amount of change and that it has the added advantage of simultaneous clarification. At first we used Seitz filters which worked well but proved to be not entirely suitable for quantity production. They occupy much space, are expensive and the filtering surface is relatively small. We, therefore, adopted the Berkefeld filter of "N" porosity which can be obtained as large candles, 10 in. in length and 2 in. in diameter, each of which furnishes 66 sq. in. of filtering surface.

*Characteristics of a 50 per cent solution of dextrose.* Our observations have been limited to a 50 per cent solution since we have restricted our output to that strength. This solution after filtration is water-clear, colorless in quantities of 100 cc. and only slightly yellowish in 2-liter lots. If the first portion of a lot of solution shows some brown color after filtration, it is due to dextrose allowed to remain in the filter after the previous use and caramelized on autoclaving. When greater care is used in cleaning the filter, no more than a trace of this color is apparent.

The pH of the freshly made solution is usually 6.0 to 6.2. The suction used to aid filtration removes much of the dissolved  $\text{CO}_2$ , so that the filtered solution has a pH of 6.6 to 7.0. Upon standing the acidity increases so that we have seen a pH of 4.5 after 17 months and 4.4 after 20 months. This change in reaction is accompanied by no change in clarity and but little in color. We attempted to prevent this change by adjustment of the pH beforehand and, within limits, were successful (table 2). But the addition of alkali hastens caramelization and the flasks to which the

largest amounts of sodium bicarbonate had been added were deep yellow after the expiration of 6 weeks to 2 months.

A relation of pH to reactions has been disputed. Williams and Swett<sup>77</sup> added a buffer before autoclaving and after doing so had no more reactions. Stoddard<sup>41</sup> says a solution more acid than pH 6.5 is likely to produce reactions and if more acid than pH 5.5 is quite toxic. He admits that explanation on the acid-base standpoint is "difficult or impossible" since the change would be easily handled by the buffering power of the blood and thinks some other toxic substance may be developed at the same time as the acidity. He added a phosphate buffer solution just before

TABLE 2  
FIFTY PER CENT SOLUTION OF DEXTROSE WITH VARYING QUANTITIES OF SODIUM BICARBONATE

pH BEFORE FILTRATION	pH AFTER FILTRATION	pH AFTER ADDITION OF NaHCO <sub>3</sub>	pH AFTER 10 DAYS	pH AFTER 21 DAYS	pH AFTER 44 DAYS	pH AFTER 64 DAYS
6.2	6.4	*	6.2	6.2	6.2	6.0
		7.1	6.7	6.5	6.2	6.0
		7.3	7.0	6.7	6.4	6.1
		7.8	7.3	6.9	6.7	6.1
		8.1	7.8	7.3	6.9	6.7
		8.3	8.1	7.8	7.3	7.3
		8.4	8.4	8.4	8.0	7.6

\* None added.

administration. He had no more reactions and speculates upon the possibility that phosphates have some detoxifying action.

Ingersoll<sup>18</sup> recommends neutralization of dextrose solution before sterilization. Hartman<sup>15</sup> and Palmer, Turner and Gibb,<sup>31</sup> however, maintain that no adjustment of the pH is necessary. Seibert<sup>37</sup> and Banks<sup>1</sup> were unable to produce reactions in animals by injection of solutions of pH from 3.8 to 9.2. Rademaker<sup>32,33</sup> in his excellent reviews says that salines of an alkaline pH are more likely to produce reactions. Alkalinity does not occur in dextrose solutions.

In our experience we have not been able to trace any reaction to the pH of the solution. One lot of 50 per cent dextrose be-

came contaminated with molds and yeasts. It was refiltered and had a pH of 5.3. The contents of about 40 to 100 cc. flasks were injected into patients without the occurrence of a reaction. Upon two occasions flasks more than one year old with pH of less than 5.0 were used without reaction. We have repeatedly, especially during the summer months, issued flasks more than 2 months old in which there is considerable tendency to acid reaction and have seen no unfavorable results. Therefore, one is justified in concluding that pH has little to do with the production of reactions. Whether it is desirable to inject an acid solution into a patient already suffering from an acidosis is quite another question.

Contamination of the solution with a mold or a yeast occurred at times when we sterilized the 125 cc. flasks by dry heat. When we autoclaved them these contaminations practically disappeared. When the contaminant was a yeast, a faint turbidity was discernible after the 4th to 6th day at room temperature, but if it was a mold, growth was frequently not visible until the 8th to the 14th day. For this reason we keep our solutions for an observation period of two weeks or more before issuing them in order to prevent the possibility of a patient receiving a product contaminated with molds or yeasts. Strict instructions are given that no flask showing any growth or the presence of particles is to be used.

It had long been known that solutions of galactose exhibit bactericidal activity and that concentrated solutions of any sugar are not suitable for bacterial growth. Cultures made regularly from our finished product have never shown any growth of bacteria. We therefore investigated the action of 50 per cent U. S. P. dextrose upon several organisms. Regular 100 cc. flasks were inoculated in pairs. One loopful of a 24 hour broth culture was transferred to each flask in the case of the streptococci. With the other organisms 24 hour broth cultures were adjusted with sterile broth to approximately equal density, one loop was transferred to 20 cc. of sterile saline and then 1 cc. of this dilution inoculated into each flask. The flasks were shaken and platings made as indicated. Readings were recorded after incubation at 37°C. for 48 hours. The results are shown in table 3.

It is recognized that the time period for killing may vary with different strains of organisms even of the same species and that it may be influenced by the dose of inoculum. In another experiment in which massive doses were inoculated, a strain of *Escherichia coli* remained alive for 8 days and *Staphylococcus aureus* for 9 days.

These experiments show clearly the bactericidal power of 50 per cent U. S. P. dextrose applied to the vegetative forms of the commoner pathogenic bacteria. Its effect upon spores of *B.*

TABLE 3

	IMMEDIATE PLATING			FIRST DAY			SECOND DAY			THIRD DAY			FOURTH DAY		
	1.0 cc.	0.5 cc.	0.1 cc.	1.0 cc.	0.5 cc.	0.1 cc.	1.0 cc.	0.5 cc.	0.1 cc.	1.0 cc.	0.5 cc.	0.1 cc.	1.0 cc.	0.5 cc.	0.1 cc.
<i>Staph. aureus</i> I.....	475	242	60	29	11	4	2	3	1	3	0	0	0	0	0
II.....	507	252	36	20	16	1	1	0	0	0	0	0	0	0	0
<i>Escherichia coli</i> I.....	506 + s	246 + s	20 + s	0	0	0	0	0	0						
II.....	488 + s	238 + s	52 + s	0	0	0	0	0	0						
<i>Eberthella typhi</i> I.....	559 + s	242 + s	53 + s	0	0	0	0	0	0						
II.....	642 + s	277	69	1	1	1	0	0	0						
<i>Strept. viridans</i> I.....	756	329	92	0	0	0	0	0	0						
II.....	482	281	53	0	0	0	0	0	0						
<i>Strept. hemolyt.</i> I.....	501	450	122	0	0	0	0	0	0						
II.....	470	250	115	0	0	0	0	0	0						
Control I.....	0	0	0	0	0	0	0	0	0						
II.....	0	0	0	0	0	0	0	0	0						

s = spreader.

*subtilis* and *B. anthracis* was also investigated. Two drops of suspensions of broth cultures of these organisms showing spores microscopically, were inoculated into 50 cc. of 50 per cent dextrose and allowed to stand at room temperature for varying periods up to 40 days. Transplants into broth tubes after this interval showed growth in most instances, indicating that the spores had not been killed. Certain differences from the typical growth of the organism suggested that the spores may have suffered some injury. When transfers were made from broth culture to broth culture at short intervals so as to avoid the formation of spores,

and the last culture treated with 50 per cent dextrose, cultures made from this mixture after four days showed no growth. These results indicate that 50 per cent dextrose is unable to kill spores of *B. subtilis* or *B. anthracis* but does kill vegetative forms of these organisms in four days.

This experiment demonstrates one of the advantages of the use of 50 per cent dextrose as a stock solution. While the rate of killing varies somewhat with different strains, a solution kept for two weeks before issue to the clinical services may confidently be regarded as free from any living vegetative forms of bacteria. The likelihood of contamination with spores of pathogenic organisms is negligible so long as care is used in handling the solution. The yeasts and molds are, therefore, the only organisms which need concern us and the efficiency of our method is shown by the fact that during the year 1935 when about 10,000 flasks of this solution were prepared, no instance of contamination with mold or yeast or any other organism occurred.

It should be reported that in our 8 years of experience we have seen but one instance of infection following the use of this solution.

This was an emaciated woman suffering from mammary carcinoma with metastases. Radical operation for removal of both breasts and contents of the right axilla was performed and immediately thereafter she was given a hypodermoclysis of 5 per cent dextrose in physiological saline, 1050 cc. in 20 minutes. The same day she received a blood transfusion. On the following day she grew steadily worse and complained of pain in the thighs, the site of the hypodermoclysis. She received a second clysis and died within a few hours. Culture from the subcutaneous tissue revealed the presence of *Cl. Welchii*. An investigation revealed that some technical difficulties arose during the administration of the clysis which necessitated a change from the needles that were being used and the new needles obtained had not been sterilized. The patient was in a very serious condition when the clysis was given and clinically the infection was rather mild so it is doubtful whether it contributed appreciably to her death.

Since the only organisms that need be considered as contaminants in 50 per cent dextrose are yeasts and molds, an attempt was made to modify the method so as to prevent their growth. An alkaline reaction was produced by the addition of sterile solution

of sodium bicarbonate. Flasks were adjusted to pH 7.1, 7.3, 7.8, 8.1, 8.3 and 8.4 respectively. Eleven such series of flasks were inoculated, nine with nine varieties of yeasts and two with molds, a *Penicillium* and a *Mucor*. We found the effects of the alkaline reaction were greatly to inhibit or even to prevent the growth of most of the yeasts. At pH 8.4 seven of the nine varieties of yeasts showed no growth after 25 days at room temperature while at pH 8.3 and 8.1, growth was either absent or scanty. Growth of the molds was somewhat inhibited but did not take place in all flasks. Moreover, caramelization of the sugar was marked in most flasks. Since these additions failed to render the medium unsuitable for the development of molds and some yeasts, and the addition of alkali complicated the procedure, we abandoned this method.

Several other methods including treatment with an adsorbent for removal of nitrogenous food material, were tried without success and we concluded that only by use of strictly aseptic technic could we assure ourselves of a sterile product. The addition of a small amount of disinfectant, such as cresol, had been a frequent practice in the past and when the solution was administered in small doses, did no obvious harm. When much larger doses were given, it became possible for the patient to receive a poisonous dose of the disinfectant<sup>42</sup> beside other possible disadvantages and the practice was gradually discontinued. We have never used any disinfectant in our solutions and, so far as we know, none is used in dextrose solutions today.

*(To be continued)*



## SUPRAVITAL OBSERVATIONS ON SOME UNCOMMON INTRACELLULAR STRUCTURES OF THE CELLULAR ELEMENTS IN HUMAN PERIPHERAL BLOOD\*

LOWELL A. ERF

*From the Laboratories of the Mount Sinai Hospital, New York, New York*

The application of the supravital technique to the study of intracellular structures reveals characteristics of red and white blood cells and of platelets which are otherwise frequently indiscernible. Although many dyes can be used, neutral red and Janus Green are the ones most frequently employed for this purpose (Sabin<sup>1</sup>). The former stains the leucocytic granules and vacuoles while the latter is specific for the mitochondria. Whereas neutral red is only slightly toxic to the leucocyte, Janus Green is very potent in this regard.

Normal leucocytic granules vary in size from those that are relatively invisible to those found in myelocytes which occasionally measure as much as a micron in diameter. Since neutral red is an acid-base indicator from pH of 6.8 (yellow) to a pH 8.0 (maroon), the hydrogen ion concentration of the leucocytic granules can be estimated.

The leucocytic vacuoles which are stained by neutral red and vary in size (from 0.1 to over 3 microns) are of approximately the same hydrogen ion concentration in most leucocytes. The clasmotocytic vacuoles, however, vary markedly in their staining characteristics, and the monocytic vacuoles are more acid than the polymorphonuclear and lymphocytic vacuoles. Non-staining vacuoles are found infrequently in leucocytes.

The mitochondria which are made obvious by the use of Janus Green are either respiratory structures (Cowdry<sup>2</sup>) or digestive enzyme producers (Koehring<sup>3</sup>). They vary in both size and

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shape. The monocytic mitochondria are thin rods (ca. 0.1 microns by 0.5 microns) while mitochondria of lymphoid cells (infectious mononucleosis) are large and ovoid (ca. 2 microns in diameter).

The supravital technique enables one to differentiate various types of leucocytes by the size, shape, motility (within the cytoplasm), color and degenerative characteristics of the normal intracellular structures. This report shall concern itself with the characteristics of some of the rarer intracellular structures observed in pathological states as revealed by the supravital technique.

#### RED BLOOD CELLS

The normal red blood cells are devoid of intracellular structures. However, the normal immature or reticulated cells contain mitochondria which are often seen in active Brownian movement. In some abnormally matured red blood cells, Howell-Jolly bodies may be seen as single or multiple colorless glass-like spheroids. They vary in size from 0.5 to 3 microns and exhibit Brownian movement. Since nuclear material does not stain in supravital preparations and since these bodies act in a similar manner it is assumed that they are nuclear fragments. They occur most frequently in the red blood cells after splenectomy, in splenic vessel thromboses and severe anemias (e.g., pernicious anemia). Another type of nuclear residua is that seen as faint, colorless, "figure of eight" shaped lines known as Cabot rings. Stained preparations reveal these particular structures more distinctly than supravital preparations.

#### WHITE BLOOD CELLS

Besides the normal intracellular structures of the white blood cells, many abnormal intracellular structures may occur in pathological conditions. In four cases of myeloid leukemia we have observed the so-called Auer Bodies. These bodies stain deeply with neutral red. Degenerative changes such as vacuolization, do not occur in them, even after prolonged observation in supravital preparations. This is in agreement with the observation of Goodwin<sup>4</sup> who found that Auer Bodies do not degenerate

even if the cells that contain them are severely traumatized. Their characteristic motion, staining properties and lack of degenerative changes lends support to the hypothesis that they are closely related to pre-neutrophilic granules.

In one case of atypical myeloid leukemia we have observed opaque colorless spherical granules in the cytoplasm of the neutrophilic and eosinophilic myelocytes. In the fixed sections and stained smears these structures appear as vacuoles whereas in the supravital preparation they are observed as distinct bodies. This difference may be attributed to the fact that their refractive index is similar to that of oil or balsam and that, therefore, they can be seen as distinct bodies with the high dry lens; the oil immersion revealing only vacuoles. (Bohrod<sup>5</sup>.) They vary in size and resemble fat droplets but are not stained with Sudan III, Wrights, Giemsa, Nile Blue, iodine nor by Mallory's phosphotungstic acid technique. Similar structures are observed in Gaucher cells, both within the cytoplasm and between the fibrils. Such granule-filled Gaucher cells are known as "foam cells" but the significance of these intracellular structures is unknown.

About 2 per cent of the peripheral lymphocytes from three cases of lymphosarcoma revealed glass-like refractile granules in supravital preparations as was observed by Wiseman<sup>6</sup>. These small, whitish, highly refractive granules are found only in the lymphocytes of such cases. It is doubtful that these granules are similar to the refractile bodies described by Gall<sup>7</sup> in 30 per cent of normal lymphocytes.

Of value in distinguishing monocytes from myelocytes is the identification of granules or vacuoles. This is readily performed by allowing neutral red supravital preparations (without Janus Green) of blood to remain at room temperature from three to twelve hours. This results in the development and enlargement of the vacuoles in the monocytes; the granules in the myelocytes remaining unchanged. If permanent records of these vacuolar changes are desired the cover slip may be removed at any time and the slide or cover slip stained according to the wishes of the observer.

## PLATELETS

The examination of platelets in supravital preparations requires alacrity since the platelets normally disintegrate in from one to three minutes. Degeneration of the platelets starts with the development of small bubbles near the periphery of the cytoplasm of the platelet. These bubbles gradually enlarge until they break loose and flow free in the plasma. They vary in size from 0.5 to 5 microns in diameter and contain small granules which have a very rapid motion of an independent or Brownian type. As early as 1874 Osler<sup>8</sup> discussed this disintegration process which he said consisted of "projecting filaments that break off" until "the surrounding area becomes alive with moving forms" which he felt were not in Brownian motion. Red blood cells will frequently fragment when included in a mass of these degenerating platelets (Hayem<sup>9</sup>). Such platelet changes can be seen in refracted light in view of the fact that dark field examinations are easily accomplished with supravital preparations. (Hausen-Pruss<sup>10</sup>.)

## SUMMARY

Such studies make it obvious that the supravital technique is of great value in distinguishing the morphological and functional characteristics of the intracellular structures which are not apparent in other types of preparations.

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## A MODIFICATION OF THE HINTON TEST APPLIED TO SPINAL FLUID

JOHN A. V. DAVIES

*From the Wassermann Laboratory, Harvard Medical School, and the Department of Communicable Diseases, Harvard School of Public Health*

The recent evaluation of serodiagnostic tests for syphilis conducted by the United States Public Health Service included an examination of the spinal fluid by both the flocculation and the complement fixation methods.<sup>1</sup> The sensitivity and the specificity of these tests varied considerably. The Kahn flocculation test on the spinal fluid appeared to lead all the other methods with a sensitivity of 92.5 per cent and a specificity of 100 per cent. Although the Hinton test on blood serum was 6 per cent more sensitive than the Kahn test and only 0.7 per cent less specific no reliable flocculation method has as yet been developed by Hinton for application to the spinal fluid. The methods here described represent an effort to supply this lack. They embody several new features but, in their essentials, are modifications of the Hinton test,<sup>2</sup> since they employ the regular Hinton "indicator" or antigen.

A possible reason for this delay in developing a reliable Hinton test on the spinal fluid is the relatively low concentration of specific reacting substance in most spinal fluids. This assumption is consistent with the normally low concentration of globulin, complement, agglutinins and precipitins in such material. Kahn<sup>3</sup> overcomes this difficulty by concentrating the globulins of the spinal fluid by ammonium sulphate precipitation. For the Wassermann complement fixation test on spinal fluid, the amount of the latter employed is several times that of the serum required for a Wassermann test.

If only the regular Hinton indicator or antigen<sup>2</sup> is used to test spinal fluid, the resulting test is relatively insensitive as compared



with the Wassermann reaction. When, however, various colloidal substances, such as egg albumin, human serum, 5 per cent gum ghatti, 30 per cent gum acacia, or 10 per cent gelatin, are added in proper proportions to spinal fluid, the sensitivity of the test is markedly increased. Although a 5 per cent solution of gum ghatti in distilled water and 30 per cent gum acacia with 4.5 per cent NaCl\* gave comparable and definite increases in sensitivity, it was found empirically that the further addition of Hinton-negative non-syphilitic human serum, inactivated at 56°C. for 30 minutes and passed through a Berkefeld filter, sharpened the results appreciably.

The human serum was obtained in pooled lots of 50 cc. and was preserved in sterile rubber-stopped, small glass bottles. After a month or six weeks, certainly after three months, there is a definite degree of deterioration of the serum for the purposes of the test, and a new lot must be prepared. Cow, horse, rabbit, and guinea pig serum, human placental extract, egg albumin and glycerine were unsatisfactory. Cow and horse serum induced a high percentage of false positive results. The best proportion for the acacia-serum mixture was found to be equal parts of 20 per cent gum acacia† (or 5 per cent gum ghatti) and Hinton-negative human serum.

#### FIRST MODIFICATION

##### *Procedure*

Two test tubes measuring 1 cm. x 10 cm. are set up for each spinal fluid.

Tube No. 1 contains 0.6 cc. of spinal fluid plus 0.2 cc. of acacia-serum plus 0.2 cc. of Hinton indicator.

Tube No. 2 contains 0.6 cc. of spinal fluid plus 0.2 cc. of acacia-serum plus 0.6 cc. of Hinton indicator.

The contents of each tube are well mixed by shaking and placed in a water bath at 37°C. for 16 hours. They are then centrifuged at high speed for five minutes and read directly. If more than two or three spinal fluid specimens

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\* 100 cc. ampoules of 30 per cent gum acacia with 4.5 per cent NaCl were purchased from the Eli Lilly Company.

† Two parts 30 per cent gum acacia with 4.5 per cent NaCl diluted with 1 part normal saline.

are to be examined at one time, the 20 per cent gum acacia-serum mixture may first be added to the proper proportions of Hinton indicator and these ingredients then mixed with the spinal fluid, thus not only saving time, but also minimizing inaccuracies in measurements. Controls consist of normal salt solution, with acacia-serum and indicator added in the proper amounts.

TABLE 1  
COMPARATIVE RESULT OF THE AUTHOR'S MODIFIED HINTON TEST AND THE  
WASSERMANN REACTION

RESULTS OF TESTS		NUMBER OF CASES
H+*	W+†	447
H-	W-	427
H±	W±	4
Disagreement‡		87
Total.....		965

\* Author's modified Hinton test.

† Wassermann reaction.

‡ See table 2.

Key: Positive = +, Negative = -, Doubtful = ±.

TABLE 2  
ANALYSIS OF CASES IN WHICH THERE WAS DISAGREEMENT

RESULTS OF TEST		HINTON CORRECT	WASSERMANN CORRECT	CASES NOT INVESTIGATED	TOTAL NUMBER OF CASES
H+*	W±†	5	0	1	6
H+	W-	13	1	7	21
H-	W+	1	4	0	5
H+	W±	25	1	10	36
H±	W+	0	1	0	1
H±	W-	5	0	1	6
H-	W±	2	5	5	12
Totals.....		51	12	24	87

\* Author's modified Hinton test.

† Wassermann reaction.

Key: Positive = +, Negative = -, Doubtful = ±.

### Readings

A positive reaction consists of clearing of the liquid and flocculation throughout or at the meniscus in either tube. Negative reactions preserve the original ground-glass appearance in both tubes. Doubtful reactions may sometimes

be rendered more definite by re-centrifuging for five minutes.\* When the contents of contaminated, turbid tubes give negative reactions, they are reported as "Unsatisfactory."

#### EVALUATION

In order to evaluate this modification of the Hinton test, 965 spinal fluid specimens were examined by this method, with the results shown in tables 1 and 2. Contaminated specimens were not included in the tabulation unless they gave definitely positive readings. The spinal fluid specimens were obtained from the Massachusetts State Wassermann laboratory and had been kept in the refrigerator for from one to five days, on the average, after the Wassermann tests had been performed. Where discrepancies arose (see table 2) between the modified Hinton and the Wassermann tests, an attempt was made to investigate the source of the spinal fluid and to determine the neurological diagnosis, together with confirmatory blood tests or previous spinal fluid Wassermann tests. A doubtful test was adjudged correct whenever a patient with neuro-syphilis had had treatment, or whenever the neurological diagnosis was questionable but the patient had other evidence of syphilis.

#### DISCUSSION

Analysis of the tables shows that the two tests agreed in over 90 per cent of the specimens examined, which were about equally divided between positive and negative tests. Moreover, this modification of the Hinton test was more sensitive than the Wassermann test. Information was obtainable from 63 cases where discrepancies arose between the two tests, and in 51 cases, or 81 per cent of these cases, the modified Hinton test was found to be correct. There was only one definitely false positive Hinton reaction. The test appeared particularly useful in cases giving a doubtful Wassermann reaction.

In addition to its greater sensitivity and accuracy, this modification embodies the advantages of flocculation tests in general.

\* Subsequent experience has demonstrated the importance of re-centrifuging for five minutes all tubes which give negative or doubtful initial readings. A small number of these tubes, for reasons as yet not clear, give positive readings after a second centrifuging. The speed of the centrifuge is approximately 1800 r.p.m.

The Hinton-negative human serum used in the test may be obtained from the serum discarded after performing the routine serological tests of the laboratory, selecting the clearer specimens. It requires Berkefeld filtration and bottling under sterile precautions in small lots. Serum obtained from a donor under sterile precautions of course does not require filtration. As mentioned above, it will keep in the refrigerator for at least one month.

#### SECOND MODIFICATION

A second modification, which appears to give thoroughly specific and possibly slightly more sensitive results, involves the precipitation of the globulins in the spinal fluid with a saturated solution of ammonium sulphate (2) (4). In each of two centrifuge tubes 1 cc. of spinal fluid and 1 cc. of a saturated solution of ammonium sulphate are mixed, incubated in a 37°C. water bath for fifteen minutes, and centrifuged at high speed for fifteen minutes. The supernatant fluid is carefully drawn off with a capillary pipette and discarded. The precipitate is dissolved in 0.1 cc. of a mixture of two parts of normal salt solution and one part of the special 20 per cent gum acacia-human serum mixture described in the first modification. To one tube 0.1 cc. of indicator is added, and to the other 0.5 cc. of indicator (antigen). The contents of each tube are mixed separately by shaking and the tubes then incubated in a water bath at 37°C. for 16 hours. They are then centrifuged at high speed for five minutes and read directly, with the usual criteria for positives and negatives. Controls consist of normal salt solution (instead of spinal fluid). The tests need not be read as closely as in the case of unprecipitated spinal fluid preparations, since the particles in a positive reaction tend to be larger. Although no significant series of tests employing the method of ammonium sulphate precipitation can be offered at this time, our limited experience indicates that the method is practicable. It involves, however, several more steps than the first modification.

#### SUMMARY

Two methods are presented by which modifications of the Hinton flocculation test for syphilis can be performed on spinal fluid specimens.

In a series of 965 tests on spinal fluid specimens by the first modification, the results appear to be somewhat more accurate and sensitive than the Wassermann reactions on the same specimens. The first method is recommended because of its greater simplicity.

Acknowledgment is hereby made of the valuable assistance of Miss Genevieve O. Stuart. Dr. William A. Hinton has offered helpful suggestions.

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## THE EXAMINATION OF CEREBRO-SPINAL FLUIDS BY COLLOIDAL CARBON\*

WM. J. DEADMAN, F. J. ELLIOTT AND H. SMITH

*From the Department of Pathology, Hamilton General Hospital, Ontario*

The examination of cerebro-spinal fluids by means of colloidal reagents has for years assumed an important place in laboratories carrying out serological tests. Colloidal gold, one of the earliest to be used, still occupies a leading place, but its use is fraught with certain difficulties; its preparation is intricate and somewhat hazardous; its keeping qualities vary, and it is difficult to be sure that successive lots have the same sensitivity, while the reading of the reaction calls for a fairly keen color sense. The introduction of colloidal mastic and colloidal benzoin obviated the color difficulty and gave a more easily prepared and preserved reagent. Several other colloidal reagents have come into use, and the latest one is colloidal carbon, which, while it is not yet perhaps firmly established, seems to have some advantages over others, particularly with regard to ease of preparation, keeping qualities, and ease of reading the reaction.

In 1927, Looney<sup>1</sup> reported that a colloidal carbon prepared by electrolysis of soft carbon electrodes in a 0.03 N solution of chromic acid would keep, and could be used in the examination of cerebro-spinal fluid in a manner somewhat similar to that used in connection with other colloidal reagents. Later Looney and Stratton<sup>2</sup> gave the method of preparation in more detail and reported the use of the colloidal carbon thus obtained, in testing spinal fluids, in addition to a method of regulating its sensitivity. Schube and Harms<sup>3</sup> suggested the use of a colloidal carbon reagent prepared by using a 1 per cent solution of Carter's Black India Ink, No.

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358, instead of that secured by electrolysis. They also reported the results of tests on 100 spinal fluids, and concluded that positive tests had a definite relation to the presence of neurosyphilis. Schube,<sup>4</sup> in a later report, suggested the use of four tubes instead of six, except in cases of meningitis, where the fluid is unsterile. The results of the examination of 552 fluids was given. Our experience suggests that four tubes are ample for all but a very small percentage of cases.

TABLE 1

NO.	WASSERMANN		COLLOIDAL CARBON	COLLOIDAL MASTIC	COLLOIDAL BENZOIN	COLLOIDAL GOLD	DIAGNOSIS
	C. S. F.	BL.					
397	++++	++++	300 000	444 432			Neuro- syphilis
336	++	+++	440 000		123 333 333 211		"
290	+++	+++	430 000	444 300		333 431 000 0	"
232	+++	+++	400 000	433 100		344 310 000 0	"
241	++++	++++	431 144	444 322	444 443 342 211	444 444 321 1	"
228	+++	+++	400 000	443 100	123 333 333 200		"
220	+++	+++	440 000		123 333 233 310		"
190	+++	+++	430 000	444 311			"
145	++++	++++	440 000	444 441			"
127	++++	++++	430 000	444 444			"
114	+++	+++	440 000		333 333 300 333		"
88	+++	+++	400 000	444 320	133 444 444 410	444 443 324 2	"
71	++++	++++	320 000	344 444			"
42	++++	++++	431 144	444 322	444 443 342 221	444 444 321 1	"
427	+++	+++	300 000		002 133 333 310		"
392	+++	—	320 000	332 100			"
283	++++	—	300 000	444 444			"
217	+	—	400 000		000 003 333 320		"
153	++	—	400 000		000 003 333 331		"
14	+	—	300 000		112 212 333 322		"

About 200 specimens of spinal fluid pass through our laboratories a year for examination. Routine Wassermann reactions are done on all specimens submitted, and if quantity of fluid permits, colloidal gold, colloidal mastic, colloidal benzoïn, and colloidal carbon tests are carried out, in addition to other routine tests as indicated. The majority of the specimens come from the hospital and are relatively fresh, but a certain number are sent in

TABLE 2

NO.	WASSER-MANN		COLLOIDAL CARBON	COLLOIDAL MASTIC	COLLOIDAL BENZOIN	COLLOIDAL GOLD	DIAGNOSIS
	C. S. F.	Bl.					
10	—	—	000 000	000 000			Hydrocephalus
43	—	—	000 000	000 000			Encephalitis
193	—	—	000 000	022 100	000 004 420 000		Multiple sclerosis
354	—	—	000 000	122 100	001 034 344 430	000 233 210 0	Fractured skull
329	—	—	000 000	111 000		012 221 000 0	Hysteria
402	—	—	000 000	222 100	000 004 124 431	111 133 200 0	Concussion of brain
289	—	—	000 000	110 000	111 123 444 420	223 444 100 0	T. B. meningitis
122	—	—	000 000	000 000	000 003 442 000	112 211 000 0	Cerebral hemorrhage
360	—	—	000 000	000 000	000 003 444 300	001 222 100 0	Glioblastoma
393	—	—	000 000	000 000	000 023 331 000	002 210 000 0	Meningitis (strept.)

## Fluids containing blood

152	—	—	000 000		000 033 233 333		Pachymeningitis, hypertension
157	—	—	000 000		000 001 023 030		Rheumatic fever
178	—	—	000 000	000 000	000 004 441 000		Cerebral hemorrhage
196	—	—	000 000	033 333			Cerebral hemorrhage
169	—	—	000 000	000 000			Cerebral hemorrhage
388	—	—	000 000	000 000			Hemorrhagic meningitis
396	—	—	000 000	000 000			Fractured skull
260	—	—	000 000	000 000	000 444 332 100	022 321 000 0	Cerebral hemorrhage
333	—	—	000 004	444 442			Cerebral hemorrhage

by outside physicians. All samples are kept on ice, after arrival at the laboratory. We have used colloidal carbon tests now on over 400 specimens of cerebrospinal fluid.

Our technique has followed closely that of Schube and Harms. We have used a 1 per cent colloidal carbon made by diluting 1 cc. of Carter's India Ink, No. 358, to a total volume of 100 cc. with distilled water. Dilutions of spinal fluid are made in six tubes, with distilled water, as follows;  $\frac{1}{2}$ ,  $\frac{1}{4}$ ,  $\frac{1}{8}$ ,  $\frac{1}{16}$ ,  $\frac{1}{32}$ ,  $\frac{1}{64}$ . A seventh tube contains 1 cc. distilled water, and acts as a control. To each of the seven tubes is added 0.1 cc. of a 0.1 per cent solution of oxalic acid, and finally 0.4 cc. of the colloidal carbon is added. The tubes are well shaken, and read after standing 12 hours at room temperature. In reading the reaction, we have graded the degree of precipitation of the carbon from 0, which means no change, to 4, which means complete precipitation of the carbon.

The following tables show the results in selected groups of cases. It will be noted that in all but two of the cases, four tubes would have been sufficient. The extra work, however, in putting up the two extra tubes is not prohibitive. Table 1 shows the correspondence of the test with other tests in neurosyphilis. Table 2 shows the lack of interference by blood in the fluid, gross blood, of course, being centrifuged out before the performance of the test.

Throughout our series, we have used a colloidal carbon prepared according to the technique of Schube and Harms, from Carter's Black India Ink, No. 358. We tried several other India inks, but found them unsatisfactory as to keeping qualities. The simplicity of preparation of the reagent and of the technique of its use are points in favor of the test. The results in cases of neurosyphilis correspond closely with those of the Wassermann and other colloidal tests. Blood in spinal fluid does not seem to interfere as it does in the case of other colloidal reagents. Some of our fluids were from one to three days old, and while we have not checked this, we are of the impression that the age of the fluid, so long as it remains sterile makes little, if any, difference, to the test. Another point noticed was that readings of the reaction after standing 48 hours on the laboratory table had not changed. It is proposed to report later a series of experiments on

the effect on the test of varying degrees of blood in the fluid, and of the effect of the age of the fluid on the results of colloidal carbon tests.

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## A CRITICISM OF LABORATORY ROUTINE IN MODERN INSTITUTIONS OF PATHOLOGY\*

NATHAN CHANDLER FOOT

*From the Department of Surgical Pathology, Cornell University Medical College and New York Hospital, New York City*

There is undoubtedly a neglect of the more modern methods of laboratory procedure in many of the pathological laboratories throughout this country. As one journeys about through American and Canadian laboratories, how often, for example, does one see anything but hematoxylin-eosin sections made from blocks fixed in formalin? The fact that this is the easiest, most reliable and most deeply-rooted technique in general usage does not imply that it is necessarily the best, nor that it is all-embracing in its scope. It merely means that it is likely to cause less trouble in the laboratory and makes for a more peaceful laboratory economy. There is need for propaganda for the newer methods and it is with this in mind that this paper is presented. It is proposed to take up the various steps in preparing material for microscopic examination in the laboratory of pathology and thus attempt to demonstrate that there are a number of methods quite neglected, although in many instances they are superior to the ordinary procedures.

### FIXATION

Probably most of us are making concessions to the hydrogen-ion concentration of our formalin by keeping it over marble chips. It was not very long ago, however, that formalin solutions were made up to 10 per cent and kept in large carboys until they were exhausted, without any attempt being made to keep them neutral. Probably the most glaring and wide-spread error in the technique

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of fixation, however, is the not uncommon practice of throwing huge masses of tissue into a small amount of 10 per cent formalin and expecting them to become fixed throughout.

Mallory's two postulates, that any fixation will penetrate only about 2 mm. or so beneath the section surface, and that the fixing fluid must be large in quantity to provide a sort of mass action, seem to be largely neglected. If tissue be cut into slabs not over 4 mm. in thickness and dropped into an abundance of fixing fluid, they will be fixed from surface to surface and good blocks may be obtained from any portion of them. The area of these slabs may be immense, but if they are not over 4 mm. thick they will provide good blocks from any portion selected. The practice of dropping entire tumors into formalin and then cutting blocks from their interior at a later date is quite hopeless. The resulting sections may show a margin of well-fixed tissue, but the center is usually largely autolyzed and quite unsatisfactorily stained. It seems quite elementary to say that tissue should be as nearly alive as possible and its component cells killed and fixed as rapidly as possible in order to get good microscopic pictures, but one finds so much material that has been fixed in bulk (when the more central portions die slowly and may not become fixed at all) that this seems to be a fact commonly overlooked.

The choice of a fixing-fluid is simple, if one knows what one wishes to do with the fixed material. Some time ago I fixed normal femoral nerve in four groups of fluids and then impregnated the sections cut from the various blocks in an identical manner, using a modified Ramon y Cajal method.<sup>1</sup> The results were interesting, as four totally different pictures were obtained. One group comprised alcoholic solutions: alcohol, a modified Carnoy's solution, alcohol-formalin, etc. The second took in the various formalin fixations: plain formalin, neutral formalin, Kaiserling I, etc. The third included various chromium solutions such as Zenker's, Helly's and the like, while the fourth was made up of Bouin's solution, which contains picric acid, formalin and acetic or trichloroacetic acid. It was evident that some of these fluids introduced coagulation artifacts but the question was: which fluid gave the most nearly normal pictures? Alcohol



brought out excellent detail as it removed the lipins and cleared the nerve sheath, formalin tended to produce the nodal swellings of the axone that have often been considered pathological, but which are pure artifacts in this case. The chromium group produced a complicated dendritic effect in the myelin sheath, seen in one of the other groups, while Bouin's solution brought out the finer connective-tissue and sympathetic elements, but rendered the axone so large and pale that one doubted that it could have been quite as sturdy in its unfixed condition. The result of this experiment has led me to do the same in connection with routine material in the surgical laboratory of pathology and the outcome of this mild form of research has shown that there is a remarkable difference in the pictures one obtains while using the same staining technique, but varying the fixative.

When one has routine material that must be run through quickly, the use of formalin-alcohol (10 per cent formalin in 95 per cent alcohol) is indicated. It dispenses with the slower dehydrations and produces much less shrinking than one might suppose. Combined with a mechanical tissue-changer, this enables one to have paraffin sections in a short time, so that reports on satisfactory material may be forthcoming in 24 hours. If the tissue be that of a tumor or other specimen that promises to be complicated and difficult of diagnosis, it is always well to fix small blocks in at least two other fixatives—say Zenker's and Bouin's. The latter is a favorite in the histologic laboratory and, perhaps for this very reason, has been comparatively little used by American pathologists who stick to formalin or the chromium group. If a specimen comes from the central nervous system and one suspects that the demonstration of neuroglia may be important, one should always fix some of it in brom-formalin, (15 per cent neutral formalin with 3 per cent ammonium bromid). This assures a block that can be impregnated by one of the Spanish silver methods. Nothing is more annoying or disappointing to the pathologist than the discovery that he has unearthed a tumor of nervous origin and has no appropriately fixed block. Secondary bromuration of formalin-fixed tissue is possible, but usually inferior to the primary fixation in a fluid con-

taining ammonium bromid. The same thing holds true of material which may require anilin dyes for the demonstration of granules, for without the chromium salts one is much handicapped in doing any of the Romanowsky stains. Here again, secondary "Zenkerization" of material in a paraffin oven may help but it is inferior to primary fixation in a fluid containing potassium bichromate. This is also true of tissue to be examined for tubercle bacilli with carbol-fuchsin.

In silver impregnations simple formalin fixation will give good nuclear detail and bring out reticulum; fixation in Bouin's fluid (as shown by Laidlaw<sup>2</sup>) will reverse the impregnation so far as the nuclei are concerned, leaving them quite unstained or only slightly so, while the cytoplasm and its pseudopods are brought out very clearly. In such cases the nuclei may always be stained with hematoxylin or some other nuclear dye used as a counterstain. This is invaluable in the study of Schwann cells, for example. In either case reticulum comes out well. If one wishes to subordinate the reticulum and bring out the neuroglia fibers, one must have bromids in the fixative. This will result in the reticulum being quite unobtrusive, while the neuroglia comes out sharply. All this depends upon the initial fixative—the use of formalin alone will preclude one's obtaining good results in every instance. It has been noticed that brains fixed in formalin, or a formalin-containing solution, do not take a typical Masson trichrome stain, the neuroglia and connective tissue staining the same. If Bouin's solution is used, the connective tissue will stain blue or green while the neuroglia comes out violaceous.

#### EMBEDDING

Another fluid we have borrowed from the histological laboratory is dioxane, which is diethylen-dioxid. Fixation in alcohol-formalin followed by a few changes of dioxane will dehydrate tissue rapidly and render it suitable for immediate passage into paraffin. The blocks thus embedded cut evenly and well and the tissue remains remarkably soft and pliable, which is of great importance when one is dealing with the tough fibromas, leiomyomas and goiters of the surgical laboratory. Dioxane is miscible

on the one hand with water, on the other with paraffin, which explains its value. Compare the simplicity of such embedding with the complexity of the usual routine ascent through alcohols of increasing percentage and the changes of chloroform, or xylol. The dioxane may be kept anhydrous by keeping a little calcium oxid in the bottom of the beakers. As this crumbles it may be filtered out and fresh quicklime added. Bucher<sup>3</sup> recommends calcium chlorid for this purpose.

A common difficulty in paraffin work is the shredding and crumbling of the sections cut from the tougher specimens. Again the histologists, this time in zoological laboratories, have supplied us with a paraffin mixture superior to pure paraffin. By mixing 100 grams of paraffin with 20 grams of crude rubber, melted and stirred in over a hot-plate, one obtains a medium that is very cohesive, does not tend to crumble and softens less in hot weather than does pure paraffin. By mixing 4 to 5 parts of this with 100 of paraffin and adding 1 part of beeswax, a mixture is obtained that may be used the year around (Hance<sup>4</sup>).

The electrical or other mechanical refrigerator has proved to be very useful in paraffin work. The ice-cubes from one of these machines are always available, handy and present flat surfaces against which paraffin blocks may be held and chilled before cutting. Or blocks may be stored in the refrigerator for a time and chilled that way. Plenty of ice on hand when paraffin cutting is to be done will lighten the work of the technicians enormously.

#### SECTION CUTTING

The Minot type of rotary microtome is intended for the cutting of ribbons of sections which may be floated on hot water and cut apart with a hot knife before floating them onto slides. The water may be kept at the desired temperature by placing a pyrex pie dish on an electrical hot plate provided with three degrees of heat. The process is simple and it is eminently suited to mass-production, where class sets are to be made, yet many technicians still cut one section, float it onto a drop of cold water on a glass slide, heat the slide to flatten the section and evaporate the water and then dry the mount on a hot-plate. This is a real "horse-

and-buggy" method, consuming much time and giving no better results than the much more rapid Mallory method of floating whole ribbons of sections on hot water and cutting them off as wanted with a hot knife. A rotary microtome may be purchased for approximately one month's salary of the ordinary technician and will pay for itself in a very short time, through increased efficiency. The sliding microtome is essential for celloidin sections, but wastes time on the paraffin technique.

#### FROZEN SECTIONS

A word might be said about frozen section technique. If one be in a hurry, there is no need to use a microtome at all. Terry's<sup>5</sup> method of cutting thin slices of fixed material (brought to a boil in neutral formalin) and painting the upper surface with a suitable stain, is extremely satisfactory and rapid. The section is cut with an ordinary razor to about 1 or 2 mm. thickness, pressed firmly onto a glass slide to prevent the dye's getting at the under surface, painted over the upper surface with a camel's hair brush dipped in Harris' hematoxylin or a polychrome methylen blue solution and after rinsing off the stain with a medicine dropper of water, the cover-slip is placed on top of the section. As the dye penetrates about 5 or 10 microns after a few seconds or a minute or so, the effect is like that of a frozen section. The material must be cut thin enough to allow transmitted light to filter upward through it and illuminate the stained surface.

Many methods are given for making rapid frozen sections, but they are usually open to the same criticism: the unfixed material makes sections that curl into tight tubes, or are readily torn, or stick to the blotting paper, or vex one in a hundred ways. This is conducive to nervousness and uncertainty on the part of the pathologist. Frozen sections that rival paraffin sections may be obtained in five or ten minutes with the technique of Mallory and Wright,<sup>6</sup> published many years ago, but not as universally used as it deserves to be. Sections are blotted onto slides smeared with egg-albumin-glycerin, using at least four thicknesses of filter paper. Ninety-five per cent alcohol is then dropped over the section followed by absolute alcohol and very

thin celloidin, so thin that it drops readily from a dropping bottle. The process is then reversed, absolute alcohol and 95 per cent alcohol are dropped on and the slide placed in water, after which it is stained in the usual way with hematoxylin and eosin and mounted in balsam, which renders the sections permanent and capable of being stored as records. When examining one of these sections the pathologist is on familiar ground, the stain is good and the diagnosis correspondingly reliable. Surgeons have been found more than willing to wait a few minutes for a definite diagnosis, rather than to have a questionable one, that needs confirmation, after a few seconds. While sections are staining in hematoxylin and eosin, a polychrome methylene blue section may be stained under a cover-slip and examined as a preliminary, by dropping on a drop of the dye and placing a cover-slip over this drop, without any other formalities.

#### STAINING

When it comes to a discussion of staining methods a volume might be written on the subject; several have been but there are many pathologists who seem to be quite ignorant of that fact. (See Lee,<sup>7</sup> Mallory and Wright,<sup>8</sup> McClung,<sup>8</sup> Schmorl.<sup>9</sup>) The tried-and-true, not-to-be-despised or under-rated hematoxylin-eosin technique dates back to the gray dawn of pathology; it still remains the pathologists' only love in many instances. The result is that we have a predominantly red-and-blue outlook on pathologic histology in the average laboratory and are quite lost if somebody switches the color-scheme to blue-and-red, not to say green, orange and brown. This should not be so, we should be morphologists first and secondly colorists. The literature of the "H and E" method is mostly of the vintage of the early 80's—over fifty years old. In the hands of the tyro, nothing can be more fool-proof and satisfactory, but its diagnostic advantages are decidedly limited. Hematoxylin stains practically nothing but the nuclei, eosin stains everything else as well as these, so that the stain is quite unselective. Usually this suffices for easy diagnoses, but it falls lamentably short of perfection when we wish to know something about details. In our laboratory we



have supplanted it in every branch but frozen sections and rapid smears, by Masson's trichrome method (Masson,<sup>10</sup> Foot<sup>11</sup>), using the light green variant rather than the aniline blue. This is merely a question of personal preference, we feel that the complementary colors are less taxing on the eye. There is nothing absolutely new or at all formidable about this method, it is merely an improved Mallory connective-tissue stain, as Masson has pointed out in his papers on the subject. In fact, there is very little new in pathologic histologic methods, most of them merely representing improvements on older ones. Masson has improved the hematoxylin-eosin technique by offering his hematoxylin-phloxin-saffron method; he has given us two color schemes for the Mallory connective-tissue technique, one rendering this tissue in blue as in the original, the other in green, and he has an improved Van Gieson technique that makes the original seem drab in comparison. The advantages of these trichrome methods are that one orients one's self much more easily if the connective-tissue boundaries are distinctively stained and readily recognized. The use of Regaud's iron hematoxylin for the nuclear stain insures excellent nuclear detail. The green, or blue dyes are valuable in staining mucus, immature thyroid secretion (colloid) and the like and in leaving nerve sheaths and muscle alone so that they take distinctive red colors and, in the case of a leiomyoma for example, fairly leap out from the green or blue background of collagenous tissue. One may thus estimate the amount of fibrosis or scarring at a glance. One may also immediately recognize nervous tissue in a neurofibroma and differentiate it from the fibrous. The method is very valuable in staining sections of brain, or brain tumors, differentiating connective-tissue from neuroglia very beautifully if the proper fixation has been employed. The new men, coming on service in the laboratory, require about a week or ten days to get used to the new color-scheme and when they return to red-and-blue they reject it as inferior.

These stains require good sections and a little more time and care than does hematoxylin-eosin, but the technician soon learns the routine steps and turns out the slides without difficulty. We have been instrumental in introducing this technique into several



laboratories in New York, where it has become the regular routine.

The accurate diagnosis of some of the diseases of lymph nodes, such as Hodgkins' granuloma, the leukemias and lymphosarcoma requires good Giemsa or other Romanowsky techniques. It is in the realm of tumor diagnosis that special stains other than routine hematoxylin-eosin or Masson, become imperative. There are a host of techniques for the demonstration of mucus, fat, hemosiderin, glycogen and the like and it is humiliating to find how few pathologists have recourse to their use. The pathologist must know these methods and be able to instruct his technician in their use, otherwise he is at the mercy of his technical staff who may produce very misleading results with an unfamiliar method.

The silver impregnations, all built up on two general basic methods, are of great importance in diagnosing certain tumors and lesions of the central nervous system. Ramon y Cajal has given us the ancestor of those methods employing silver nitrate and a photographic developer, Bielschowsky that of those in which double salts of silver and ammonia are used, such as the diammino oxid or carbonate, with formalin as the developer. The former are chiefly of use in the staining of nerve fibers and cells, the latter in the demonstration of neuroglia cells and fibers. The latter method is also of importance in demonstrating reticulum as opposed to collagen, in connective-tissue. Many mistakes have been made in the interpretation of impregnations of nervous tissue with salts other than silver nitrate, for reticulum fibers, incompletely impregnated, take on bizarre forms which lead to more bizarre interpretations.

One might wonder wherein lies the importance of demonstrating reticulum and consider this merely an academic question. Certain tumors produce it, others do not; some merely distort the preexisting reticulum, whilst others produce a new network of their own. This is of value in diagnosing retothelial sarcoma, for example, where a luxuriant new reticulum is produced by the retothelial cells. There is a form of lymphosarcoma in which the type cell occupies a position partway between retothelial and

lymphoid cells; it produces large alveoli or masses of tumor cells so closely resembling epithelial cells that one is often perplexed as to whether it is a primary tumor of the lymph node or a metastatic carcinoma. The reticulum, in such tumors, penetrates the cell masses and runs among their cells; in the carcinoma it becomes compacted into a sort of basement membrane and penetrates only a short distance into the cell masses. This may not be absolute proof, but it is a valuable diagnostic point.

Most of the modern advances in neuropathology have been made by the silver route. The central nervous system had been revealing itself to us very grudgingly until the silver methods were applied by Ramon y Cajal, Bielschowsky, and others. How can one expect to get anywhere in the unravelling of the mystery surrounding tumors of the nervous system if one sticks stubbornly to hematoxylin-eosin? A glioblastoma multiforme looks much like a fibrosarcoma under these circumstances; the ependymomas, oligodendrogliomas and the like have come into recognition only since silver impregnations showed their characteristic intricacies. Some pathologists prefer to leave the diagnosis of these tumors to neuropathologists, but many of these specialists devote most of their time to clinical work and have a correspondingly limited perspective in things pathological. Why leave the pathology of any human organ out of consideration in the practice of pathological diagnosis? The placing of the melanomas in the category of neurogenic tumors is a result of study through special stains, it has become accepted by its most stubborn antagonists who, until Masson used special methods, had been misled into believing these tumors to be "droppings off" from the basal epithelium of the epidermis. With hematoxylin-eosin the basal cells and the tumor cells appear to be identical, with special stains, however, they become quite different from one another.

#### ASPIRATION BIOPSIES

These come into the laboratory in two forms: (a) Those taken by aspirating solid plugs of tissue through a syringe needle and expelling these onto slides, where they are crushed and smeared. (b) Centrifugated fluids from the thorax, abdomen, or other body cavities. These may be in considerable quantity (Foot<sup>12</sup>).

Much may be learned from both types of aspiration biopsy. The smears are usually stained with hematoxylin-eosin, whilst the fixed, centrifugated sediments are embedded in paraffin and sectioned in the usual manner, after which they may be stained by any desired method. There is a tendency to doubt the veracity of smeared aspirations, as one has no anatomical relations to go by, there is more or less distortion of the cells, and so on, but the fact remains that one learns to judge these results empirically and to make few mistakes, if we may trust experience gained through the examination of many prostatic aspirations.

As for the centrifugated sediments, they are of real value in the diagnosis of neoplastic conditions of the various body cavities. One may find whole fragments of tumor, in which case the diagnosis is positive and simple, or one may find discrete cells of a nature manifestly neoplastic and foreign to the locality whence they came. In this case the diagnosis is again simple. It is when the cells resemble desquamated mesothelium, or macrophages, that one encounters difficulty. Apparently much of this may be circumvented by resorting to a determination of the ratio between the diameters of the nuclei and their nucleoli. When this is done figures are obtained that are diagnostically accurate in over 60 per cent of the cases, if one relies upon them alone without considering any other helpful criteria; one may expect 80 per cent or better of accurate diagnoses if one uses all the criteria at hand, including the all-important history. A much neglected bit of technique lies right here—clinicians fail to supply a case history when submitting specimens. Embarrassing situations are often avoidable if one knows the status and habits of the patient.

#### MACHINERY

In this machine age one might expect to find more mechanical contrivances in the laboratory, but they gain ground very slowly. There is a mechanical tissue-changer that works while the technician sleeps and has the day's material in paraffin, ready for blocking and cutting when she comes in the following morning. This is a real boon to laboratories where rapid and prompt diagnosis is important. The machine is not cumbersome and its occasional vagaries are readily dealt with by the house electrician.

Diagnoses in bone pathology depend largely on satisfactory decalcification, which in turn depends on having the bone in thin blocks that can be easily penetrated by the acid. A mechanically driven band-saw is a great advantage in this respect for thin sections may be cut in a twinkling, entire femora bisected and prepared for museum specimens, calcified goiters and leiomyomas may be dealt with in the same way. A hand-saw demands a good deal more skill in this connection.

Opinions differ as to the applicability of portable electric circular saws in the necropsy room. Success or failure in their use depend much upon the mechanical skill of the user, be he professor or diener. They reduce the time necessary for opening skulls, channeling the vertebral column for removal of the spinal cord and similar procedures. One must guard against their becoming entangled in fibrous tissue or muscle and running wild. It is advisable to use a disc on the saw limiting the depth of section. This will, for instance, permit the cutting of a groove around the calvarium that is deep enough to enable one to finish the job with a few taps of the hammer and chisel. It prevents the saw from becoming a source of danger to the operator; although he might get a nasty cut, he could not lose a finger!

Rotary microtomes are usually provided with channeled fly-wheels all ready for a belt from an electric motor. One seldom sees one mechanically driven, however, as the average technician prefers the added accuracy afforded by hand-cranking. That the motor would save much time or add to the efficiency of the microtome, seems doubtful.

#### CONCLUSION

In conclusion, this paper has been somewhat rambling and informal, but I have meant to make it so. It is hoped that some of the points brought out in it will be of value and possibly stimulate some interest in advancing laboratory technique and drawing upon its rich resources.

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## A FLOCCULATION METHOD FOR THE DIAGNOSIS OF ACTIVE TUBERCULOSIS\*

F. RYTZ AND G. K. HIGGINS

*From the Clinical Laboratories of the Minneapolis General Hospital, Minneapolis, Minnesota, and the Department of Pathology, Glen Lake Sanatorium, Oak Terrace, Minnesota*

In the detection of activity in tuberculous lesions, clinicians have been guided by various means and methods, including X-ray findings over a shorter or longer period of time in individual cases of tuberculous infection. A simple serological reaction, able to differentiate with reasonable accuracy, and aiding in the detection of early active tuberculosis, should therefore prove to be of value.

In 1923 Larson<sup>1</sup> reported a ring-test for the diagnosis of tuberculosis, and in 1934 Meinicke<sup>2,3</sup> described a specific antigen and a diagnostic test for that disease.

The test to be described is apparently of a nonspecific nature as a simple alcohol-saline mixture will serve as an antigen. However, it has been shown by Lehmann-Facijs<sup>4</sup> and Steinert<sup>5</sup> that in tuberculous infections the euglobulin may acquire antigenic properties and combine in vitro with the antibody. On that basis it would be explainable why the present reaction and other tests may become negative in severe cases of active tuberculosis where great numbers of disintegrated tubercle bacilli and other cell substances possibly enter the circulation to combine with the antibody of the blood stream. The free antibody of the circulation, ordinarily causing a reaction, may thus be combined and unable to unite in vitro with antigenic protein, or other antigenic substances, possibly activated by alcohol-saline solution.

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*The antigen*

For the present method an antigen was prepared from ground tubercle bacilli (H37) which had been grown on glycerol-agar. Two grams of bacilli were placed in 5 per cent phenol for 12 hours, then washed once in distilled water, transferred to a mortar with a few cubic centimeters of alcohol, and ground for 30 minutes. The bacilli were then placed in a bottle 2 x 2 inches, containing 4 cc. of alcohol, and ground under the weight of small steel balls, covered by somewhat larger glass beads, by agitating in a mechanical shaker for 5 hours. Microscopic examination proved the grinding to be nearly complete. To the ground material was added 75 cc. of 95 per cent alcohol. This suspension was left at room temperature for 3 days, and then filtered through a very dense filter paper. A clear extract resulted which by complement fixation tests was proved to have antigenic properties.

For the flocculation reaction it was found that 1 part of the extract to 3 parts of saline gave the best results. In attempting to establish the required concentration of the extract, it was found that a very low concentration gave the same results as a highly concentrated alcoholic extract. Finally, 95 per cent alcohol without any bacillary substance, diluted with saline in the proportion of 1 part of alcohol to 3 parts of saline, gave the same results as the tubercle bacilli extract diluted in the same proportion. The majority of serums were tested by both methods, and it was found that the alcohol, and not the bacillary substance, was of main importance in the reaction. A few serums showed a slightly heavier reaction in the tube with the extract-saline mixture, and, occasionally, the alcohol-saline solution gave a more distinct flocculate. If possible, it would therefore seem advisable to use 2 tubes for each serum, 1 with extract-saline, and 1 with alcohol-saline mixture.

*The serum*

The serum for this test must be free from hemolysis and blood cells, and the sample should not be over 2 days old. The blood sample should remain corked up from the time of collection until performance of the test. If the blood has been stored for a longer period of time, an atypical precipitate may form in positive serum, but flocculation usually does not take place. All glassware should be thoroughly rinsed out with distilled water before being placed in the drying oven, as minor traces of acid or alkali may cause a positive serum to give a negative reaction.

*The test*

1. If a tubercle bacilli extract, as described above, has been prepared, a dilution is made in the proportion of 1 part of extract to 3 parts of saline. This is shaken vigorously for a few seconds. In a second tube an alcohol dilution is made in the same proportion; 1 part of 95 per cent alcohol to 3 parts of saline. This is also shaken vigorously. If no extract has been prepared, the alcohol-saline mixture may be relied upon for readable results.

2. Clear serum is obtained by centrifugation.
3. The serum unit, 0.3 cc., is measured into the respective tubes, the size of which should preferably be 10 x 75 mm.
4. The rack holding the tubes is placed in a water bath at 60°C. for 5 minutes.
5. After the serums have cooled completely, 0.3 cc. of the diluted extract (or diluted alcohol) is added to each tube. This is mixed by shaking the rack lightly, and the tubes are then left standing for 1 minute.
6. The rack with the tubes is placed in the Kahn shaking apparatus and agitated for 10 minutes. (If shaken by hand, the rack should make 275 oscillations per minute.)
7. The tubes are read over the Fisher lamp for reading Kahns, or a similar light convenient for the purpose, preferably aided by a hand lens. A positive reaction consists of distinct floccules, evenly distributed in a more or less hazy fluid. A negative test has a clearer fluid without floccules.

#### ANIMAL EXPERIMENTS

There is evidence that this test is positive in very early tuberculosis. In tuberculous guinea pigs the test is negative except for a few days after inoculation. Four pigs were injected subcutaneously with small doses of a very weak suspension from a culture of H37. On the 5th day after inoculation, blood samples were collected from the hearts of the animals, and the flocculation test was positive in all instances. Six control pigs gave negative reactions. A week later the infected animals also gave negative tests. At the same time the glands of the test pigs had become slightly enlarged. Six weeks later the test pigs were killed. One animal developed an abscess at the site of inoculation, and from this animal tubercle bacilli were recovered both from the abscess and from the lungs. The lungs, liver and spleen from the remaining 3 pigs showed miliary tuberculosis. Repeatedly the serum from these guinea pigs gave negative tests except for the blood samples drawn a few days after inoculation of the tubercle bacilli.

#### RESULTS IN HUMAN TUBERCULOSIS

By this method 860 serums have been tested. Of that number 175 were from patients in tuberculosis sanatoriums. By clinical classification 121 were from patients with active tuberculosis, including cases of lupus, bone tuberculosis, empyema and tuber-

culosis of the larynx. Of that group 39 were clinically defined as borderline cases. Blood samples were also taken from 54 tuberculous patients, clinically classified as arrested. Of that number 49 gave negative reactions. In a comparatively small number of severe and hopeless cases of active tuberculosis, the test had become negative in about 50 per cent.

In 80 per cent of the total number within the group of tuberculous patients, the serological findings agreed with the clinical classification.

A comparatively large number of blood samples, 685, from presumably nontuberculous patients admitted to the Minneapolis General Hospital, served as negative controls. Of that group 21 or 3 per cent gave positive reactions: 1 per cent due to early active tuberculosis, 1 per cent from elderly patients (above 69 years of age), 1 per cent from patients with miscellaneous disorders, alcoholism, pneumonia, colds, etc.

The mechanism of false positive serological reactions is generally little understood. Unaccounted-for protein changes have been shown to influence various serological tests.<sup>6</sup> By this method the serum from the same patients with tuberculous infection constantly gives positive tests. The reaction, however, is not constant if due to other causes, except in the case of old age. Examination of more than 1 blood sample from each patient is therefore of importance. The few cases in which the test may be positive due to colds or pneumonia, generally become negative within 2 weeks following convalescence. In cases where old tuberculin has been applied to the skin, this test may be weakly or atypically positive for several weeks. In a few instances the reaction has been found positive due to acute alcoholism, but such patients showed negative tests a few days after alcohol consumption had ceased.

#### SUMMARY

A flocculation method for the detection of active tuberculosis has been described. For this method a specific antigen is not required. It has been shown that this test gives comparatively few false positive reactions and that it is of value in the detection

of early active tuberculosis. In the great majority of patients, clinically defined as arrested, this method gives negative reactions. In severe and hopeless cases of tuberculosis this test may become negative.

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- (4) LEHMANN-FACIUS, H.: Antigenspezifität der Serum-Euglobuline als Grundlage einer neuen Serodiagnostik von Krebs und Tuberkulose. *Klin. Wchs.*, **12**: 333. 1933.
- (5) STEINERT, R.: Praktische Versuche mit der Seroreaktion nach Lehmann-Facius und Loeschke zum Nachweis präcipitierende Antikörper by aktiver Tuberkulose. *Beit. zur Klin. der Tubek.*, **68**: 545. 1928.
- (6) LEHMANN-FACIUS, H.: Die serologische Bedeutung der Globulinveränderung. *Zeitsch. f. Immunitätsforsch. u. Exper. Therap.*, **46**: 137. 1926.

## PART II

### ABSTRACTS OF PUBLICATIONS ON NECROPSIES, 1931 TO 1934 (Continued)

Prepared by Committee on Necropsies of the American Society  
of Clinical Pathologists for the year 1934-1935.

I. DAVIDSOHN, M.D., *Chairman.*

A. HELWIG, M.D.

O. SAPHIR, M.D.

M. WARWICK, M.D.

*Necropsies on exhumed bodies.* (Enterdigung und Sektionserfolg) F. Klemp. Deutsch. Ztschr. f. d. ges. gericht. Med., 16: 190-209, 1930. The author discusses 24 necropsies performed at the Institute of Legal Medicine in Leipzig following exhumation. The gastro-intestinal tract resisted most the post mortem changes and putrefaction, then the lungs and the heart. Organs of the neck, the liver, spleen, and the kidneys underwent marked post mortem changes in a short time. The non gravid uterus and the brain remained fairly well preserved for a long time. Klemp emphasizes the extent of information that can be obtained by examination of exhumed bodies.—I. Davidsohn.

*Necropsy method to correlate X-Ray and necropsy findings.* F. Windholz. Virchow's Arch. f. path. Anat., 277: 658-669, 1930. Windholz discusses a special method which was developed for the purpose of correlating x-ray and necropsy findings. The usual necropsy procedure, by removing the organs from the skeleton, destroys their topographic relationship to bony structures. Other objections to removal of the organs are destruction of cavities, displacement of interlobular and encapsulated exudates, etc. The author claims for his method a further application of the maxim of Albrecht: to destroy nothing, including the relationship of organs, so long as there are significant findings. The incision starts over the acromial third of the left clavicle, continues across the interclavicular notch, over the right clavicle and terminates about 12 to 15 cm. to the right of the sternoclavicular joint. From this point a curved incision extends to the middle of the right axilla, and from there straight down to the anterior superior iliac spine where it bends and runs above and parallel with Poupart's ligament to the symphysis pubis. In its course on the chest, the incision penetrates skin and muscle, but on the abdomen, only skin and fat tissue. The sternoclavicular joint is then disarticulated. Through the straight

portion of the incision, just below the costal margin a small opening into the abdominal cavity is made and any free peritoneal fluid evacuated and measured. This opening is then extended by cutting through the entire thickness of the abdominal wall along the line of the above described incision.

The left rectus muscle is then cut from the inside. The ribs on the right side are cut along the anterior axillary line with a rib shears. The presence of pneumothorax is determined and liquid contents of the chest cavity are collected and measured. All the muscles in this line are cut but the great vessels are preserved. The assistant lifts the anterior wall of the thorax and the dissector cuts the ligamentum teres hepatis, the attachment of the diaphragm to the ribs and of the mediastinum to the sternum. When necessary he carefully cuts adhesions of the pleura and the small cervical muscles of the left side. The left ribs are cut through from the inside along a line similar to that followed on the right. If necessary, the abdominal muscles of the left side may eventually be sectioned from the abdominal aspect. The anterior wall of the chest and abdomen can now be reflected, like the cover of a book. For further orientation in special cases the following procedure is advised. The inside surface of the anterior wall of the chest cavity is first carefully dried and subsequently painted with a concentrated gentian violet solution containing 19 per cent Gum Arabic. Then the wall of the chest is restored in the original position and the outlines of the ribs "printed" on the lungs and liver. This procedure produces an almost exact imprint of the topographic land marks. To restore their normal volume the lungs are inflated with air introduced through the trachea. The volume used should be about the normal 4000 cc. Further procedure depends on the individual case. Further dissection and x-ray examination can now be combined, as necessary. This method offers the following advantages: the preservation of the topographical relationships thus allowing comparison of x-ray and pathologic anatomical findings; an easy access to the apex of the lung, the lateral region of the lung and the organs below the diaphragm, while preserving the entire situs. A disadvantage is the danger of laceration of the hand by the sharp exposed ends of the ribs.—O. Saphir.

*Necropsy observations on new-born infants.* R. Beneke. Beitr. z. path. Anat. u. z. allg. Path., **84**: 551-558, 1930. Beneke recommends a method of examination by means of which the position of the infant in utero may be judged. The method consists of bending the extremities and the body in the direction of least resistance. In doing this routinely the author believes that malformations which possibly may be the result of peculiar positions of the fetus could be studied to greater advantage.—O. Saphir.

*Dermatographic examination of cadavers; determination of time of death.* Schrader. Deutsch. Ztschr. f. d. ges. gericht. Med., **16**: 256-271, 1931. Many difficulties prevent an exact dermatographic examination of cadavers. The most important factor is the condition of the skin turgor which may show considerable variations in relation to the disease and the immediate cause of death. Further factors: humidity of the air, temperature. No positive results with



regard to the determination of time of death were obtained by the author.—*I. Davidsohn.*

*Determination of carbon monoxide in exhumed bodies.* W. Weinmann. Dtsch. Ztschr. f. gericht. Med., **17**: 1, 1931. Even in the warm season, carbon monoxide may be found in the exhumed body, several weeks after death. If blood or transudate is not available, fluid expressed from internal organs, especially lungs and spleen, should be examined.—*A. Hellwig.*

*Anleitung fuer den Sektionskurs, Am. Path. Institut Tuebingen.* Dietrich and Nordmann. Franz Pietzker, Tuebingen, 1931. Twenty-two pages, intended for the medical student.—*A. Hellwig.*

*Coöperation in necropsy work.* Editorial. J. A. M. A., **97**: 1894, 1931. Percentage of necropsies performed in this country is still lamentably low. Necropsy is one of the chief means of advancement of medical knowledge. Reasons for failure to obtain permission are obvious: 1. lack of scientific interest in the physician in charge; 2. opposition of relatives; 3. objections of morticians. A joint committee of the N. Y. Academy of Medicine, N. Y. Path. Soc. and Metrop. Funeral Directors Ass. hopes to bring about coöperation between pathologists and morticians. The November issue of the Casket and Sunnyside, publication for morticians has the following note: "We might decide that if we explain our difficulties to the medical practitioners rather than antagonizing them, they will be willing to coöperate with us in avoiding unnecessary mutilation of bodies committed to them for autopsy."—*A. Hellwig.*

*Medicolegal aspect of necropsies.* O. Warneier. Chirurg., **3**: 497-501, 1931. The author, a judge of the supreme court of Germany (Reichsgericht) discusses the medicolegal aspect of necropsies.—*I. Davidsohn.*

*New official regulations in Bavaria in regard to procedure.* H. Merkel. Muench Med. Wochnsch., **78**: 2086-2089, 1931. A discussion of the new official regulations in Bavaria concerning the medico-legal necropsy.—*I. Davidsohn.*

*Criminological aspect of necropsies.* Nippe. Deutsche Ztschr. f. d. ges. gericht. Med., **18**: 103-120, 1931. The problems involved in performing a medico-legal and a regular necropsy are different. The physician who is doing medico-legal necropsies must also be, to a certain extent, a detective. The particular significance of findings on teeth, bones, eyes, hair, as well as in the urine, is stressed. The determination of time of death is an important problem.—*I. Davidsohn.*

*The necessity of a necropsy after occupational injuries.* Bergel. Ztschr. f. Med. Beamte, **45**: 165-173, 1932. Twelve cases are presented in which the death followed an occupational injury in the past (after intervals of up to fifteen years). The connection between trauma and death is discussed and the necessity of necropsies in such cases illustrated. In many cases the insurance could be refused.—*I. Davidsohn.*

*Value of necropsies.* Faugnez. An. de méd. lég., **12**: 143-148, 1932. The author illustrates on hand of three observations some strange coincidences

which obscured the real cause of death. The latter could be detected only by a necropsy. The author emphasizes the enormous natural resistance of the human body. A man 43 years old died suddenly a week following an accident (injury to the skull). Necropsy findings: 1. lobar pneumonia in the stage of red hepatisation; 2. old vegetative endocarditis (recurrent); 3. acute suppurative generalized leptomeningitis (severe form). This man died while working in his garden complaining only of a moderate weakness and headaches.—*I. Davidsohn.*

*Necessity of necropsies in automobile accidents.* Muller. *Ann. De Méd. Lég.*, 12: 232-239, 1932. The necessity of a necropsy in automobile accidents is emphasized. Only the necropsy permits to determine exactly the possible pathological condition (f.i. alcohol intoxication) of the victim.—*I. Davidsohn.*

*Necessity of necropsies after fatal occupational accidents; two cases.* R. Szumlanski and H. De Soille. *Ann. de méd. lég.*, 12: 89-95, 1932. Two cases are presented which show the necessity of determination of the exact cause of death by a necropsy in the cases of death following occupational accidents. Case 1. 31-year-old factory worker suffered an accident, was immediately taken to the hospital, expired six weeks later, the clinical diagnosis being cerebral abscess. Necropsy findings: tumor in the temporo-occipital region, histologically glioma. The author considers the cause of death fully independent from the accident. Case 2. 55-year-old man working in a garage died under circumstances suggesting that death following CO<sub>2</sub> intoxication. Necropsy revealed an acute perforation of a duodenal ulcer.—*I. Davidsohn.*

*Necropsy technique.* A. Schmincke. *Centralb. f. allg. Path. u. path. Anst.*, 53: 273-275, 1932. Schmincke discusses the removal of the brain and spinal cord in connection with one another. For this purpose he recommends an incision extending along the spinal processes of the vertebrae to the region of the parietal bone where it meets the usual circular incision made for removal of the skull cap. The skin is then dissected off the underlying muscles of the back and the occiput. Starting from the incisions over the spinal column and over the skull, the skin is drawn laterally toward the acromion. The skull cap is removed next and then the occipital bone by the following procedure: two incisions starting from points on the circular incision located about 8 cm. lateral to the posterior midline extend obliquely and converge towards the lateral wall of the foramen magnum. The bone between the incisions is then removed, exposing the dura mater, the cerebellum and medulla oblongata. The spinal cord can be exposed next according to standard methods. This method is especially recommended to demonstrate tumors in the cerebellar pontine angle, syringomyelia or primary and metastatic tumors of the medulla oblongata. The author also describes a new method of demonstrating the nasal cavity and the maxillary sinus. He recommends an incision along the clavicles extending over both shoulders and from the shoulders to the spinal column. A second incision perpendicular to this one extends along the spinal processes of the vertebrae to the region of the parietal bone. Next, the skin is reflected

anteriorly, laterally, and posteriorly. Because of the fact that the previous incision was made posteriorly along the spinal process, there is little resistance met with in the removal of the skin from the skull and neck up to the mandible. The mandible is disarticulated next and with a chisel, the alveolar processes of the maxillary bone are cut through.—*O. Saphir.*

*Necropsies of the bodies of the sovereign bishops of Wuerzburg during the 17th and 18th centuries.* A. Holzmänn. Virch. Archiv., **283**: 513, 1932. During 1684 and 1795 necropsies were done on the bishops of Wuerzburg for the purpose of embalming. Causes of death were pneumonia, typhoid fever, arteriosclerosis, prostatic hypertrophy, aortic aneurysm.—*A. Hellwig.*

*Der Neubau des Path. Instituts der Universität München.* M. Borst and Th. Kollman. München 1932. Verlag der Kunst im Druck. Beautifully illustrated booklet on the new luxurious pathological institute at München.—*A. Hellwig.*

*Sektionstechnik.* R. Roessle. Springer, Berlin, 1932. Fifty pages. Roessle's technic is a combination of Virchow's and Heller's methods. There is a model necropsy report and a table of measurement and weights of organs.—*A. Hellwig.*

*Method of dissecting the upper respiratory and digestive organs.* S. Graeff. Cbl. f. allg. Path. u. path. Anat., **53**: 369, 1932. The method allows to remove the upper respiratory and digestive organs in connection with the basis of the skull.—*A. Hellwig.*

*An autopsy table—a new design.* W. D. Forbus. Arch. Path., **14**: 505, 1932. Detailed description with drawings of author's model. The table costs \$345.22.—*A. Hellwig.*

*Preliminary report on bacteriologic autopsies in man.* L. Heerup. Acta path. scand. Suppl. 1. 1932. Under sterile conditions, blood is removed by means of Pasteur pipettes from both ventricles of the heart, jugular vein and portal vein, also urine, bile, pleural and peritoneal fluid. From internal organs and lymph nodes, small pieces of tissue are placed on blood agar and in semifluid agar. Heerup found that at least in winter time, the abdominal organs remain sterile for many hours. Putrefaction starts as a rule from the lungs. In spite of its presence pathogenic bacteria may be demonstrated successfully. Often general infection was recognized by bacteriological autopsy, while clinical and anatomic evidence was absent. In children, a general streptococcic infection may originate from the lungs, without causing definite pneumonia.—*A. Hellwig.*

*Report on necropsies.* Prepared by the joint committee representing the N. Y. Academy of Medicine, the N. Y. Pathol. Soc. and the Metropolitan Funeral Director's Association. Arch. Path., **14**: 701, 1932. (1) The hospital owes it to the family to give an account of what has occurred on the death of a patient. The funeral director should not oppose the proper efforts of the hospital authorities to obtain permission for necropsy. (2) Unreasonable delay by the hospital in its attempt to obtain permission for necropsy, is objectionable to the funeral director. (3) The funeral director must present

to the hospital acceptable evidence that he has been authorized by the family to take charge of the body. (4) Hospital employees must not give information to favored funeral directors in regard to persons critically ill or dead in the hospital. Proof that a minor employee has recommended a funeral director to the family should be followed by his instant dismissal from the service. (5) To avoid delay after death, the hospital should make certain that the necessary data for a death certificate are entered on the record at the time of admission. (6) It is improper for any member of the hospital staff to threaten to call the medical examiner if permission for necropsy is refused. Any such procedure of threatening or browbeating may be regarded as evidence of lack of ability to handle the situation. (7) In general, the permission for necropsy should be asked for as soon as possible after death has occurred. (8) The funeral director should be promptly informed by telephone when the death certificate is signed and the body is ready for him. (9) Interference by a funeral director with the legitimate efforts of the hospital to obtain permission for necropsy shall be regarded as a reportable grievance. Technic of necropsy: Y-incision. In female below breast. Ligate external and internal carotid and vertebrals. Divide scalp by incision behind the ear, passing over the vertex. Saw skull in two intersection lines which meet at an obtuse angle behind the ear. The anterior incision begins at the level of the hair line. Provide against leakage from cranial cavity. Ligation of carotid and vertebral arteries. Plugging of foramen magnum with cotton. Fill cranial cavity with oakum. Suture skin with small needle. Deliver body to embalmer in a clean condition. Wash skin, sponge and dry all cavities. Do not allow any leakage. After completion of the necropsy, allow the embalmer the use of the necropsy room for the preparation of the body for burial.—A. Hellwig.

*Das Recht zur klinischen Leichensektion (Law and necropsy).* G. Bohen. Leipzig Hirschfeld. 1932. Legal complications arising from the performance of necropsies. Able discussion of the German laws pertaining to necropsy and removal of organs.—A. Hellwig.

*Necropsies in deaths due to accidents.* Foreign letters. J. A. M. A., **98**: 1579, 1932. Fauquez emphasized the fact that after five cases of death attributed to occupational accidents, there was a case of double pneumonia, a case of thrombosis of the mesenteric vessels, two cases of cerebral tumor and one case of endocarditis with suppurative meningitis. Need of necropsy in all accidental deaths is therefore evident. Lyon Caen recalled a previous communication on two cases in which death following automobile accidents was caused in reality by cerebral tumors Leclercq remarked that often judges hesitate too long in ordering a necropsy. In civil suits, the court often hesitates for reasons of economy to order a necropsy. (Société de Médecine légale in Paris, March 1932).—A. Hellwig.

*Medical news, New York.* J. A. M. A., **99**: 1613, 1932. The state public health council at a meeting September 23 added to the sanitary code a new regulation requiring tissue removed at operation or necropsy for examination

must be submitted for examination to an approved laboratory, or to the division of laboratories and research in Albany or New York, or to the state institute for the study of malignant diseases at Buffalo.—A. Hellwig.

*Results of bacteriologic studies in 400 necropsies.* J. Putnoky. Zbl. f. Bakt. I. Orig., **126**: 248, 1932. Bacteriologic examinations are of value until the 55th hour after death. In 68.5 per cent bacteria were found in different organs. In 97 per cent of the positive cases, the entrance of the bacteria into the circulation could be demonstrated. If the patient's resistance is low a mild inflammation or circumscribed abscesses may cause septicemia. In peritonitis and after abdominal operations, pathogenic bacteria may enter the blood stream easily.—A. Hellwig.

*Anatomo-pathologic observations on post mortem material in China.* J. Heine. Virchows Arch. f. path. Inst., **287**: 203-226, 1932. Observations on 864 surgical specimens and 106 necropsies. The author believes that the difference in the frequency of the various diseases in China and Europe are more likely due to the external factors (climate, customs, food, sanitary conditions) than to the hereditary racial disposition.—I. Davidsohn.

*Necessity and possibility of further clarification of doubtful bacteriologic findings in connection with necropsies.* E. Eckestein. Muench. Med. Wchnschr., **80**: 1352-1353, 1933. Some doubtful bacteriologic findings before death can be cleared only by necropsy. The necessity of conducting more necropsies is emphasized. Public education about the importance of necropsies is necessary.—I. Davidsohn.

*Proposed changes of regulations on legal examination of the body.* F. Pietrusky. Deutsche. Ztschr. f. d. ges gericht. Med., **21**: 103-111, 1933. The author makes proposals concerning the regulations on legal examination of the body in Prussia (Germany).—I. Davidsohn.

*The first necropsies in Vienna.* (Foreign letters.) J. A. M. A., **100**: 2033, 1933. Galeazzo de Sophia, a physician of Padua, brought the art of anatomy to Vienna, where on February 12, 1404, the first necropsy was performed by him.—A. Hellwig.

*Determination of blood groups at necropsy.* G. Strassmann. Dtsch. Ztschr. f. gerichtl. Med., **21**: 2, 1933. If the body is so decomposed that serum or blood cells cannot be utilized, agglutinins may be determined in transudates, hydrocele fluid, contents of the seminal vesicles, mucus of the vagina or in the saliva.—A. Hellwig.

*Certain experiences in the postmortem room.* C. Carter. The Medico-Legal and Criminological Review, **1**: 89, 1933. Report of a case of cyanide poisoning. Smoking should be absolutely prohibited in the room where necropsies are done. There are certain odors of diagnostic importance that might be missed due to the presence of tobacco smoke.—A. Hellwig.

*Eighteenth Congress of Legal Medicine.* T. H. Blench. The Medico-Legal and Criminological Review, **1**: 272, 1933. The initial incision, as used in the Medico-legal Institute in Paris, is carried from the lower lip to the manubrium



sterni and thence round the sides of the body. The ribs are sawn through and the entire anterior aspect lifted to display the organs beneath. The skin of the lower jaw and neck is also reflected. The skull is sawn completely through above its base with a large saw, the brain being sectioned by this operation. Despite the fact that the body was decomposed, the method of refrigeration employed enabled the necropsy to be carried out with a minimum of discomfort to the operator.—A. Hellwig.

*The medico-legal importance of the examination of the blood of the cadaver.* P. Lande and P. Dervilles. Rev. med. Franc., **14**: 125, 1933. (1) Macroscopic examination (color). (2) Cytological. (3) Bacteriological. Streptococci may be identified 5 to 6 days after death, anthrax bacilli 14 days, pneumococci 3 to 4 days, Coli 2 days. (4) Chemical and spectroscopic. (5) Humoral. (6) Blood group.—A. Hellwig.

*The Autopsy. An outline of the problem.* I. Davidsohn. Am. J. Clin. Path., **3**: 199, 1933. The number of necropsies is deplorably low. Public health authorities and the government in this country have done nothing to secure for the people the undisputed and scientifically established benefits of necropsies. There is a fundamental error in the general conception of necropsies. In most parts of Germany the custom is that the relatives are notified of the patient's death and have the privilege of protesting against a necropsy during the first 24 hours. In Austria the necropsy in public hospitals is made obligatory by the law. In this country we must approach the relatives for a permission at a most inopportune time. A law should be passed making the performance of necropsies easier. Attempts without the help of public health authorities will not lead to a great success. The second major reason for the unsatisfactory results of the necropsy movement is that the medical profession while taking upon itself the selling to the public of the idea of necropsies did not sell it, first of all, to its own members. It is up to the pathologists of this country to prove the advantages of necropsies to the medical practitioners. Pathologists will have to perform the necropsy and present the findings in a less detached and academic way and will have to pay particular attention to the correlation of clinical and anatomical findings. Editors of medical periodicals could exert a great influence by not accepting papers based upon the study of conditions in which a final check-up by means of an autopsy is not included. More attention will have to be paid to the just grievances of the undertakers. A great deal of good can be done by speaking to groups of undertakers. Popular articles in lay journals may help to educate the public. Finally, the medical schools should emphasize the absolute necessity of necropsies for improving the professional efficiency of the practitioner.—A. Hellwig.

*The pathologist's duty in obtaining permission for necropsy.* W. Freeman. Am. J. Clin. Path., **3**: 211, 1933. The pathologist should take an active part in methods of stimulating an increase in the number of necropsies. He must incur and maintain the good will and confidence of all members of the hospital staff. Clinico-pathological conferences are an excellent method of stimulating



the staff to want to see necropsies and to verify the clinical diagnosis on their patients that die. The relatives in nearly every instance wish to share in the knowledge of the findings at necropsy. In the hospital of the author letters are sent routinely to the relative of every patient on whom a necropsy has been performed. The pathologist must cooperate with the undertakers. Technic: no wrist and chin ties. Y-incision. All internal organs are removed and never replaced. Their remnants are burned in the hospital incinerator. It should be avoided to leave stool in the colonic stump. Stump of rectum and vagina are tied. Vertebral and carotid arteries are tied with suture material. Upper and lower temporal edges of the cranium must be wired on either side. As suture and tie material Belfast starched white cord is used. The baseball stitch is employed. The skin is washed and wiped dry. Unnecessary delay for the undertaker must be avoided.—A. Hellwig.

*Postmortem results in alcoholism.* Queries and minor notes. J. A. M. A., 11: 443, 1933. The necropsy of a man of 45 disclosed that the brain contained 3 plus ethyl alcohol. Answer: The man was intoxicated when he died. The brain tissue contained 0.4 per cent of alcohol.—A. Hellwig.

*Regulations pertaining to necropsies and cremation.* Foreign letters, Berlin. J. A. M. A., 100: 1618, 1933. It is planned to incorporate in the new federal law uniform regulations governing cremation. The federal law would provide that necropsies may be performed only by licensed physicians. If the cause of death is not certain and if there is any suspicion that the deceased did not die a natural death, examination of the body by the sanitary police must be ordered.—A. Hellwig.

*Necropsies in accidental deaths.* G. B. Gruber. Monatsschr. f. Unfall heilk., 40: 321, 1933. Very instructive paper illustrating the fact that also in Germany the value of necropsies is underestimated by laity and physicians alike. Only experienced pathologists who are able to do microscopic work should perform these necropsies. Histological methods are often more important than gross inspection. A decision whether death was caused by the accident is often only possible by considering the clinical data. The necropsy report should be as complete as possible and should be illustrated by gross and microscopic photographs. Many case histories and autopsy reports from the experience of the writer are included. Sudden death during work is often caused by natural causes.—A. Hellwig.

*Percentage of tuberculous changes in necropsy material.* E. Winkler. Beitr. z. klin. Tuberk., 82: 655, 1933. Of 100 necropsy cases, only 2 were without evidence of tuberculosis.—A. Hellwig.

*Consent for Necropsy.* Medical News, Arkansas. J. A. M. A., 101: 54, 1933. A verdict in favor of Dr. H. S. Thatcher and his assistant was rendered by the jury in the case recently brought against them on account of a necropsy. The mother of the child on whom necropsy was performed signed a permit for it. The father was present when she did so, but he did not sign the permit. To guard against similar suits, the rule has been adopted now on requiring both

the father and the mother of a deceased child to sign a permit for a necropsy.—A. Hellwig.

*Foreign letters, London.* T. H. B. Bedford. J. A. M. A., **101**: 788, 1933. Post mortem records of the Leeds General Infirmary for the last 21 years revealed that patients dead on arrival at the hospital from causes other than trauma or suicide had in 73 per cent diseases of the circulatory system. In nineteen of the 198 cases, lesions of the respiratory apparatus were found. The central nervous system accounted for 15 cases, all hemorrhages. In 8 per cent the necropsy failed to account for death (four of these 15 cases had status lymphaticus).—A. Hellwig.

*Postmortem examinations. Method of Obtaining Them.* W. I. Hoffman. Jour. Am. Med. Ass., **101**: 1199, 1933. Necropsies for adequate instruction of students, for the development and evaluation of new methods of treatment, for the verification of diagnosis, for the accumulation of accurate statistics and for research. We must convince the laity of the need for necropsies before death, confidence in relatives should be created by good care and treatment, and it should be seen that they are there at time of death. All requests should be made by one person preferably the resident who does it in his office far from the wards and in street clothes because a white coat means immaturity to the laity. The resident should lead up to the subject gradually and talk about the case in a way to stir their curiosity. He should watch them carefully to see what approach to take, and what arguments to use. His manner must have dignity, air of responsibility and sympathy. The arguments to be used are: 1. Duty to humanity by helping develop medical knowledge. 2. Determination of exact cause of death, with reference to familial tendencies, occupational hazards, relation to former illnesses and injuries, help in collecting insurance and making more accurate vital statistics. 3. The extent of the disease should be determined. 4. Medicolegal. Best for necropsy to be done by unbiased hospital pathologist. 5. More and more people are having it done. 6. Discovery of rare diseases. 7. Aid to research. 8. Evaluation of treatment. The usual arguments are answered here: 1. "Will disfigure the body." Drains off the blood and pus. No visible evidence of it. 2. "Let someone else do it." Medical advance is at a stand-still because of this attitude. May help our own family and we should all try to save other people. 3. "Is a mutilation of the body." It is only the beginning of the inevitable decomposition which comes to every living thing. 4. "Not an unusual case." Never can tell, necropsies show many surprises. 5. "Cancer is hopeless anyway." Many are cured and we can do more about studying the way it progressed. 6. "He would not have wanted it." He would like to help others afflicted as he was. 7. "He has suffered enough." This causes no suffering and may save much suffering for others. 8. "Jewish religion." There is no real objection in this religion as rabbis will tell. Precautions to be taken: Be familiar with all the details of the case. Avoid interpreters. Make request about 15 minutes after death before the relatives get away and yet after they have recovered

from the first shock. Talk to the strongest personality among the relatives. Do not relegate it to a family friend. Do not make attempt before death. Avoid gruesome terms. "Examination of body" best. Invite family back in 2 weeks to talk it over. If there is a professional member in the family he may be asked to be present. Must leave body in good condition and not keep the undertaker waiting unnecessarily.—*M. Warwick.*

*The Autopsy Problem.* Its solution in smaller communities. G. W. Covey. Jour. Am. Med. Ass., **101**: 1209, 1933. In Lincoln, Nebraska, 3 hospitals each contributed \$50.00 a month and set up central laboratory with full time technician and part time stenographer in quarters of County Medical Society. Autopsy teams were organized, one at first and later seven, all volunteer workers. One had had pathological training and he trained the others. They did autopsies for private physicians as well as the hospitals. Three reports made and filed at central laboratory, at hospital and with attending physician. Get 15 per cent of deaths in city and 33 per cent of those in hospitals. Allow undertakers to embalm first if they do not use trocar.—*M. Warwick.*

*Importance of Clinical-Pathological Conference on the work of the practitioner as a teacher.* R. Fitz. Jour. Am. Med. Ass., **101**: 253, 1933. The American Medical Association requires 15 per cent necropsies on deaths in all hospitals for interne training because adequate training in pathology is an important qualification for residency, and a good index of the professional efficiency of the institution. Public education is the most important and more articles should be written for lay magazines. Prominent men should leave requests for autopsies. But most of the responsibility rests with the staff. Should treat patients as guests and explain all procedures to them. A necropsy should be well explained when asked for and later a letter with a card of condolence and explaining the results is sent to the family.—*M. Warwick.*

(To be continued)

## NEWS AND NOTICES

A signal honor was conferred on Walter M. Simpson, M.D. at the First International Conference on Fever Therapy held in New York in March.

Dr. Simpson, who is Director of The Kettering Institute for Medical Research at the Miami Valley Hospital, Dayton, Ohio, was made a member of the French Legion of Honor in recognition of his work in the development of fever therapy.

Arrangements for the Philadelphia convention are nearing completion. The Committee in charge are bending every effort to make this convention memorable and hope for a large attendance.

Have YOU made your reservation?

## EDITORIAL

### THE LABORATORY AND ITS FUNCTION

A recent writer has called the laboratory an adjunct to a hospital. The word adjunct means "added to but not essential." To take issue with this view may be *infra dignitatem* and in the nature of special pleading. But it can be done with self respect and there can be no quarrel with the purpose of presenting and explaining one's calling, especially when it has established its place.

No one familiar with the modern hospital and the demands of present day medical science, clinicians, practitioners of laboratory medicine and hospital executives especially, could hope to defend successfully the proposition that the intricacies of diagnosis or surgical management could be achieved without the laboratory. If this be true, as it certainly is, the term adjunct was used with the looseness of authors in this country or in the absence of knowledge of modern medicine.

Another has written that diagnoses are too often attempted in the laboratory. This is probably true and should not be done, but how often does the ward officer fail and how much does he demand of results and opinions from the laboratory?

What is more important is the management and personnel of the laboratory. The modern hospital is a teaching institution where its staff carried out routine matters and investigation; there is no real place for abstract research which is to be used when it has reached a practical form and which is always available from foundations devoted to this purpose. The laboratory is the largest and most suitable division of the hospital for both routine and investigation though clinical investigation is of very great value.

It appears not improper to define more fully the terms routine, investigation, and abstract research. Routine seems easily

definable as the application of accepted or standard technical procedures to the needs of any case. Investigation may be defined as the application of standard procedures to a group of cases or a special problem or a modification of accepted principles either of technical or clinical nature. Research is the development of a well-based theorem until it explains obscure things and results in a regular practice for investigation or routine.

The functions of a laboratory even in a small hospital comprise work in chemistry, bacteriology, serology, morbid anatomy, clinical microscopy, hygiene and liaison with the clinical side. The officer in charge should have discretion in every one of these and the success of the department depends upon his ability.

Since it is impossible for the principal officer to have anywhere near complete knowledge of every one of the above subjects, it would appear according to the standards of modern medical science, that there should be ample assistance, notably in chemistry, a subject that appears to be followed by men of rather limited interest since the subject itself appears to be conducive of single track work. Chemistry in modern medicine is too important to be done by non-medical technicians and in charge of a person incompletely trained and interested in the subject. The chemist must be of a mind to coöperate with his associates both in the laboratory and in the ward.

It appears possible for a capable man trained in biological medicine to cover the other subjects and he of course should be the executive officer. Under ideal conditions a division chief should be supplied for each of the subjects mentioned as the functions of the department. This enables the executive officer to devote his time to supervision and coöperation and enables his division chiefs to devote their abilities to the best development of the applied technical work.

It must not be forgotten in judging the laboratory officer that a very considerable part of a day may be occupied by autopsies and the work incidental thereto.

The physical work of the administration of such a department must never be ignored. Consultation with three executive



officers in laboratories reveals that from one to two and one half hours per day were needed for pure desk work.

Still another demand is made of the laboratory—the supervision of hygiene and of solutions used in the wards. These are duties that are best concentrated in one whose training has a public health and technical character and because superintendents and clinical chiefs are not militant in acquiring knowledge and discretion in them.

It can scarcely be maintained today that in the organization of the laboratory the service can be any less a distinct department than can that of surgery. Because surgical emergencies arise in a medical ward is no reason for a surgeon “adjunct” to that ward.

If it were not for the poor patients who must be treated, for which the practitioner of laboratory medicine knows he is not prepared, the clinical staff would be the adjunct service, since a modern hospital will not endure if clinicians diagnose and treat without laboratory data and opinion.

HERBERT FOX.



## THE RANGE OF HEMOGLOBIN CONCENTRATIONS AND ERYTHROCYTE COUNTS IN HEALTHY MEN AND WOMEN\*

C. FERDINAND NELSON

*From the Department of Biochemistry, University of Kansas, Lawrence, Kansas*

The concentration of hemoglobin in the blood of men and women is generally expressed today in one of two ways: Either in relative terms as the per cent of an arbitrarily chosen standard value such as, for example, those of Haldane, Williamson, or Haden, or in absolute terms as the average number of grams of hemoglobin present in 100 cc. of whole blood. Both these ways of expressing hemoglobin concentrations make use of only a single value whether the method of expressing the results be stated in relative or absolute terms. The red cell count is likewise expressed by a single average figure, although here absolute units alone are used.

The use of single average figures for expressing normal or standard values in some instances, while in others ranges are employed, is hard to understand. It is true that most of us are singularly fond of average values because we feel instinctively that the arithmetical mean represents the truth more nearly than anything else, but whether in medicine this is always true is open to serious doubt.

Many important standards employed in clinical medicine are always expressed by single values. Normal body temperature, for example, is generally considered to be neither more nor less than 98.6°, and our clinical thermometers are conspicuously calibrated to this end and thus perpetuate and keep fresh this error in the minds of both doctors and laymen.

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Body weight is also expressed as a single average value. Consider the many apprehensive and conscientious mothers, and the health-seeking adults who constantly worry and fret over the fact that they or their children are slipping from the limits of health and normality because their body weights differ from that which the tables and books tell them is right and proper and exact.

The doctor, when he is wise, looks at these tables, accepts the values that coincide with his other findings, and for all other cases makes such mental additions and subtractions as his experience dictates and lets it go at that. In instances such as these the single arithmetical mean value becomes practically meaningless because of the corrections and additions that must be introduced in order to make it coincide with facts as they really are.

In recording analytical data on urine single average values are practically always employed. It has been taught, quite dogmatically, that normal urine had a 2 per cent concentration of urea, that a 24-hour sample contained 30 grams of this substance, and that 1500 cc. was the average 24-hour output of urine; and as 2 per cent of 1500 is 30, it is all very simple and easy to learn and remember and therefore, in the interest of the practical, entirely permissible. Few textbook writers of today have taken the trouble to correct this error and explain that 30 grams is not the normal output of urea in a 24-hour sample of urine unless a high protein intake, corresponding to the Atwater-Voit standard is ingested. Creatinine is similarly given a single 24-hour value around 1.7 grams, uric acid 0.7 gram, ammonia 0.7 gram, sodium chloride 12.0 grams, and so on throughout the entire list of ordinary urinary constituents.

During the past fifteen years, and particularly since 1930, a great deal of justifiable and well-directed criticism has been leveled at the percentage method of expressing the amount of hemoglobin in normal and pathological blood. Nothing but confusion and inaccuracy can result from expressing any standard in terms of hypothetical "100 per cent dummy values," however large or small the series of normal data may be upon which these standards are based. And yet so ingrained and apparently

attractive is the percentage method of expressing hemoglobin concentrations in blood, that most doctors refuse to give it up. To call attention to the fact that 100 per cent, based on the Haldane standard differs from 100 per cent based on the Williamson standard by some 18 per cent makes apparently but little impression. They are looking to the clinical pathologists and research men to provide them with a good hemoglobin standard and, in the long run, will hold them responsible if such a standard is not forthcoming.

As a substitute for the percentage standard, to which it is manifestly impossible to adhere if accuracy and uniformity of recording hemoglobin values are to be at all considered, the method of recording hemoglobin concentrations in grams per 100 cc. of whole blood has been proposed and for some time now put into use in many laboratories. This is surely a step in the right direction, but whether it goes far enough is open to serious doubt. We do need a method of recording hemoglobin concentrations that is absolute rather than relative, but we need even more than any single absolute standard value, a standard hemoglobin range. After all, the important thing for the practicing physician to know, so far as hemoglobin is concerned, is how much and how little of this substance there is present in the blood of healthy men and women. He needs to know the limits, the maximum and minimum values, that are present in the symptomless complex which we call health so that he can truly evaluate and compare these values with the findings he obtains in disease.

I have already called attention to the many glaring faults in the percentage method of expressing hemoglobin concentrations in blood. First of all, there is no agreement as to what value shall be called a standard or 100 per cent value. In the second place, the phrase 100 per cent has no definite meaning. In blood analysis, so far as hemoglobin is concerned, it means the setting up of an average value based on a certain number of normal cases and endowing this value with the attributes of 100 per cent normality. This, all too often, implies a tacit assumption or belief that a 100 per cent value represents a per-

fect or optimum value, for quite instinctively when we say that blood has a hemoglobin value of 100 per cent the idea is generally induced or assumed that this represents perfect blood rather than average blood.

It is in the assumption, that 100 per cent represents an optimum or perfect value that fault must be found with any single standard value, whether it be stated in absolute or relative terms. The experimental data from which it is obtained makes it an average or arithmetical mean value at all times, but since it is to be used as a standard, or is called a standard value, the assumption is quite natural that it represents an optimum figure also.

A path or road is best located and defined by its limiting boundaries and not by its central line. Once these values are known the center is easy to locate. Single values, whether they be absolute or relative, can never be anything but middle-of-the-road values. They tell nothing about health at its upper and lower boundaries. Normality or health can never be expressed by any single set of values. Normality is not a narrow line—it is a wide avenue. Nature hates uniformity, individuals are never exactly alike, they all differ—grossly or minutely—but, like the finger print, differ they do. Biologically, therefore, the arithmetical mean can never represent or be substituted for the optimum. There may be, perhaps, as many optima as there are individuals, and for this reason no single average value can represent the truth, even the practical truth, for all persons. To set up, therefore, any average single value as a standard, even though it be expressed in absolute rather than relative units, is to substitute an “absolute dummy” for a relative one. Until the variables of health and disease are far better known than we know them today, we cannot pick out the perfectly normal man and woman and from them get the single value that represents perfection and the optimum. Until such time comes it seems better therefore that the maximum and minimum values compatible with health in various walks of life should be adopted as standards for hemoglobin and other substances in place of average single standard values.



We have recently completed a study of the hemoglobin concentration and red cell counts of 350 healthy men and 225 healthy women taken from widely scattered sections of our population. The series for men includes urban and suburban groups of U. S. mail carriers and mail clerks, traffic and motorcycle policemen, young men at Civilian Conservation Corps Camps, Officers and enlisted men from various branches of the regular Army, such as the General Staff and Command School, Infantry, Cavalry, Air Corps, Motor Transport Corps, and Medical Corps. For the women the series includes nurses from urban and suburban hospitals, University students majoring in athletics and in science, saleswomen from the Kresge ten cent stores, counter girls and waitresses from large cafeterias, secretaries, stenographers, and office help in metropolitan offices, club women and housewives.

The maximum and minimum hemoglobin concentrations and erythrocyte counts obtained for these groups follow:

	HEMOGLOBIN (Grams per 100 cc.)		ERYTHROCYTES (Mil. per c.mm.)	
	max.	min.	max.	min.
Men (350 cases).....	18.39	12.35	6.70	4.03
Women (225 cases).....	16.82	11.61	5.61	3.93

The hemoglobin determinations were made in duplicate or triplicate by the oxygen capacity method, and the red cell counts checked to within 100,000 cells by two and, at times, by four separate individuals. All apparatus was checked by the Bureau of Standards.

THE MAXIMUM AND MINIMUM HEMOGLOBIN CONCENTRATIONS AND  
RED CELL COUNTS OF NORMAL MEN AND WOMEN  
OBTAINED BY OTHER AMERICAN INVESTIGATORS

In tables 1 and 2 will be found the maximum and minimum hemoglobin values and red cell counts obtained in normal men and women by other American investigators during the past 15 years. The total number of cases studied for the men was 778,

TABLE 1  
MAXIMUM AND MINIMUM HEMOGLOBIN CONCENTRATIONS AND ERYTHROCYTE  
COUNTS OF NORMAL MEN, REPORTED BY VARIOUS AMERICAN INVESTIGATORS

OBSERVER	YEAR	NUMBER CASES	AGE RANGE	HEMOGLOBIN		CELLS	
				Maxi- mum	Mini- mum	Maxi- mum	Mini- mum
Haden (Detroit, K. C.) . . .	1922	20	18-30	17.17	14.02	5.62	4.52
		20	30-50	16.90	13.80	5.46	4.27
Sackett (Kansas City) . . . .	1925	15	20-50	19.30	14.98	5.92	4.70
Osgood (Portland) . . . . .	1926	137	19-30	18.63	13.44	6.40	4.41
Wintrobe-Miller (New Orleans) . . . . .	1929	100	20-30	18.30	13.40	7.53	4.68
Foster-Johnson (New Orleans) . . . . .	1931	115	18-30	18.00	13.00	6.00	4.40
Murphy, Lynch and Howard (Boston) . . . . .	1931	18	20-40	17.10	13.52	5.98	4.78
Haden (Cleveland) . . . . .	1932	15	18-30	17.31	14.72	5.45	4.70
		15	30-50	16.50	13.16	5.40	4.39
Wintrobe (Baltimore) . . . .	1933	86		18.40	13.90	5.98	4.90
Sachs, Levine and Appelsis (Omaha) . . . . .	1933	100	20-25	17.49	11.95	5.66	4.28
Walters (Lawrence, Kansas)	1934	100	20-30	18.70	12.89	5.55	4.10
Helmer and Emerson (Indianapolis) . . . . .	1934	10	20-40	16.85	14.47	6.03	5.22
Dill . . . . .	1928-36	27	19-57	17.30	13.49	5.47	4.30
Nelson and Stoker (Lawrence) . . . . .	1936	350	17-64	18.39	12.35	6.70	4.03

TABLE 2  
MAXIMUM AND MINIMUM HEMOGLOBIN CONCENTRATIONS AND ERYTHROCYTE  
COUNTS OF NORMAL WOMEN, REPORTED BY VARIOUS AMERICAN INVESTIGATORS

OBSERVER	YEAR	NUMBER CASES	AGE RANGE	HEMOGLOBIN		CELLS	
				Maxi- mum	Mini- mum	Maxi- mum	Mini- mum
Haden (Detroit, K. C.) . . .	1922	12	20-40	14.80	11.90	4.72	3.89
Sackett (Kansas City) . . . .	1925	14	20-50	17.16	13.64	5.31	4.16
Osgood and Haskins (Portland) . . . . .	1927	100	18-30	16.49	10.98	5.55	4.07
Wintrobe (New Orleans) . . .	1930	50	17-30	15.96	11.80	5.65	4.45
Murphy, Lynch and Howard (Boston) . . . . .	1931	21	20-40	15.40	12.56	5.47	4.44
Haden (Cleveland) . . . . .	1932	18	18-50	15.82	11.79	5.60	4.00
Sachs, Levine and Appelsis (Omaha) . . . . .	1933	50	20-30	14.70	9.98	4.89	4.06
Wintrobe (Baltimore) . . . .	1933	101	20-30	16.20	12.00	5.60	4.44
Helmer and Emerson (Indianapolis) . . . . .	1934	10	20-40	14.77	12.30	5.46	4.32
Nelson and Stoker (Lawrence) . . . . .	1936	225	18-60	16.82	11.61	5.61	3.93

for the women 376. The high and low hemoglobin values and red cell counts for all of these cases follow:

	HEMOGLOBIN (Grams per 100 cc.)		ERYTHROCYTES (Mil. per c.mm.)	
	max.	min.	max.	min.
Men (778 cases).....	19.30	11.95	7.53	4.10
Women (376 cases).....	17.16	9.98	5.65	3.89

Whether the maximum and minimum values recorded above represent truly normal figures is naturally open to doubt, and in the interest of conservative interpretation in attempting to arrive at standard ranges for hemoglobin and erythrocytes in healthy men and women, it might perhaps be better to include only those maximum and minimum values which have been obtained by two or more investigators. When this is done and the various figures rounded out, the following values are obtained:

	HEMOGLOBIN (Grams per 100 cc.)		ERYTHROCYTES (Mil. per c.mm.)	
	max.	min.	max.	min.
Men (1128 cases).....	18.50	12.35	6.50	4.10
Women (601 cases).....	16.80	11.00	5.50	4.00

These values it seems can at least be set down tentatively as representing the limits of hemoglobin and red cells of healthy men and women in America.

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## THECA CELL TUMOR OF THE OVARY\*

J. MARSHALL NEELY

*Lincoln General Hospital, Lincoln, Nebraska*

Löffler and Priesel,<sup>7</sup> in 1932, were the first to describe theca cell tumor of the ovary and presented six cases giving evidence that these tumors are derived from the theca interna cells of the ovary. In 1934 these same authors described four additional cases and Melnick and Kanter<sup>2</sup> in 1934, described two more cases. Kellert<sup>9</sup> in 1934 described a case of ovarian fibroma with unusual lipid change, which, judging from the description of the sections and the clinical record, was undoubtedly a theca cell tumor. Geist and Spielman<sup>1</sup> presented another case in 1935.

Geist<sup>1</sup> states that only three of the seventeen cases reported up to that time occurred before the menopause. The most constant and common clinical characteristic of theca cell tumors is uterine bleeding which may or may not be irregular. As is true of the case herewith reported, the bleeding may appear long after the menopause. There is not infrequently rejuvenation and coincident hypertrophy of the breasts. A striking similarity is seen in the clinical manifestations of theca cell and granulosa cell tumors of the ovary. In each there is evidence of excess production of estrogenic hormone.

Theca cell tumors usually become quite large before they are discovered clinically. They are solid and somewhat rubbery not unlike a fibroma and there is almost always a definite capsule present. The cut surface presents a very characteristic picture. It is a distinctly lemon yellow color. Ill defined yellowish areas are separated by interlacing bands of fibrous connective tissue which are usually quite fine although coarse bands occur. While these tumors are solid they cut with less

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resistance than the ordinary fibroma. In many instances degenerative cysts are encountered. The tumor is practically always monolateral. It may be slightly nodular but usually has a smooth surface.

Paraffin sections stained with haematoxylin and eosin present a picture not unlike that of fibroma and as in the case here reported, it is probable that some theca cell tumors may be hidden

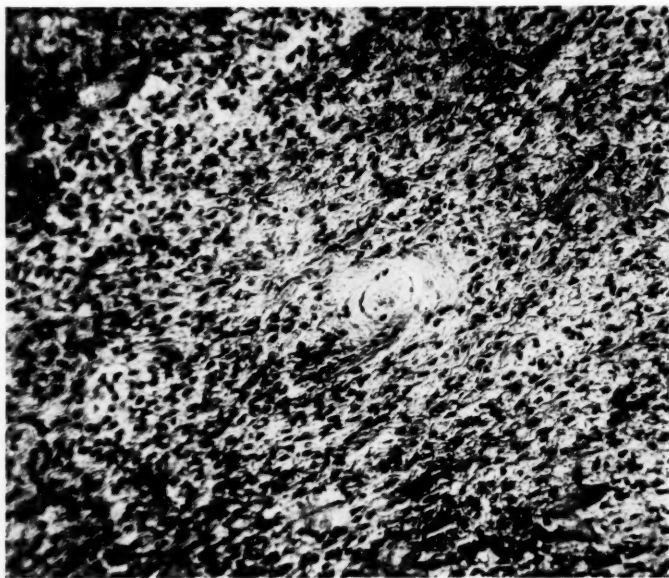


FIG. 1. LOW POWER PHOTOMICROGRAPH SHOWING CELLULAR PORTION OF TUMOR AND DEMONSTRATING INTERCELLULAR FIBERS

under the heading "Ovarian Fibroma." Careful study however, even without the aid of special stains, will usually disclose the true nature of the tumor. The tumor cells are characteristically plump, spindle shaped and, in many instances epithelioid in character. The nuclei are oval and often hyperchromatic. Many cells have varying sized cytoplasmic vacuoles. The more cellular portions of the tumor are separated by varying sized bands of collagen which in some areas have undergone hyaline degeneration. Intercellular fibrils have been described. Frozen



sections stained with Sudan III show an enormous quantity of fat throughout the tumor mostly in the cytoplasm of the theca tumor cells. In granulosa cell tumors most of the fat is in the fibrous connective tissue.

*Case Report:* The tumor here described was removed from a white woman age 70, admitted to the surgical service of the Lincoln General Hospital March 26, 1936, complaining of vaginal bleeding and low backache. She was quite



FIG. 2. HIGH POWER PHOTOMICROGRAPH OF FROZEN SECTION STAINED WITH SUDAN III

Note distribution of fat

well until three days prior to admission when, seventeen years following a normal menopause, she suddenly began to have profuse uterine hemorrhage. Physical examination disclosed a large rather freely movable mass in the right pelvis which was diagnosed an ovarian cyst. The other physical findings and routine laboratory data are unimportant.

The patient was operated upon by Drs. Harry Everett and Roy Whitham the day following admission, a complete hysterectomy, bilateral salpingectomy and oophorectomy being done.

The ovarian tumor measures 30 x 20 x 29 cm. It is firm in consistency and its external surface is smooth. On palpation it is quite rubbery in character.

The cut surface is a heterogeneous lemon yellow. There are strands of fibrous connective tissue running throughout the tumor and the intervening yellowish tissue is soft. The uterus shows a characteristic endometrial polyposis and the fallopian tubes and left ovary are not remarkable.

Several blocks were taken from the tumor, some being fixed in Zenker formol and others in 10 per cent formalin. The blocks were imbedded in paraffin,

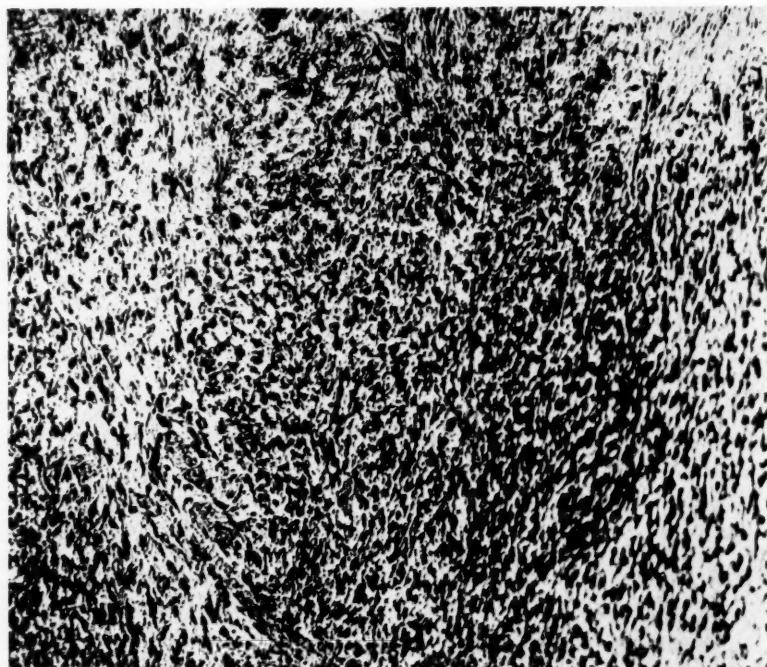


FIG. 3. LOW POWER PHOTOMICROGRAPH OF A FROZEN SECTION STAINED WITH HAEMALUM AND SUDAN III SHOWING THE LARGE AMOUNT OF FAT DISTRIBUTED THROUGHOUT THE TUMOR

Fat droplets are found both extra and intracellular

cut and stained with haematoxylin and eosin, Masson's trichrome stain, and Foot and Foot silver impregnation. Frozen sections were stained with Sudan III.

Sections stained with haematoxylin and eosin show varying sized spindle shaped cells with large hyperchromatic nuclei. In some areas there is hyaline degeneration of the collagen. No mitotic figures are seen. In the more cellular areas those sections stained with Masson's trichrome stain show definite intercellular fibrils. An occasional small blood vessel is encountered though the

tumor is quite avascular. Sections stained with Sudan III show a large quantity of fat located in the cytoplasm of the tumor cells and to a much less degree in the intercellular spaces. Unfortunately the specimen was placed in Kaiserling's solution before the true nature of the tumor was suspected and consequently hormonal studies were not done.

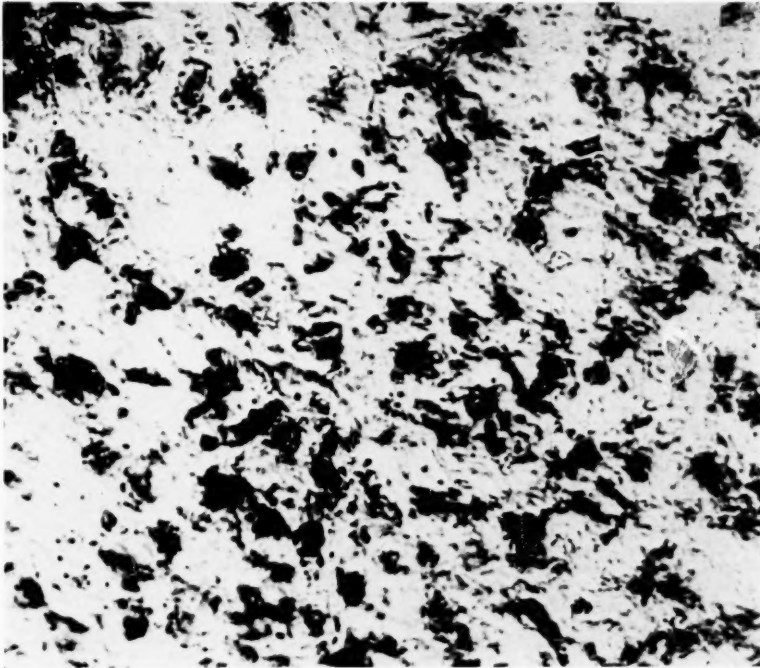


FIG. 4. HIGH POWER PHOTOMICROGRAPH SHOWING FAT DEPOSIT BOTH  
WITHIN THE CYTOPLASM OF THE TUMOR CELLS AND  
EXTRACELLULARLY

This is a frozen section stained with haemalum and Sudan III

#### DISCUSSION

Although the clinical manifestations of granulosa cell and theca cell tumors of the ovary are very similar, both the gross and microscopic characteristics of these two tumors give evidence that they represent different and distinct pathological entities. A consideration of the embryology and histology of the ovary shows that the theca interna cells and the granulosa cells

are not only closely associated with each other morphologically, but also show many physiological similarities. Both become epithelioid in character before maturation of the ovum. Also both cells accumulate lipoid droplets. After accumulation of fat the theca interna cells are known as theca luteum cells and the granulosa cells are known as granulosa luteum cells. It has been demonstrated chemically that the granulosa luteal cells contain phospholipin and the theca cells cholesterol and cholesterol esters.<sup>3</sup>

There is some difference of opinion as regards the embryological development of these two cells. Some think the granulosa cells are derived from the coelomic epithelium and others from the mesenchyme. It is quite generally agreed however, that the theca interna cells are mesenchymal in origin and it is quite apparent from the sections of theca cell tumor that cells have maintained many of the properties of mesenchyme. Sections of theca cell tumors stained with Masson's trichrome stain show definite intercellular fibrils. Their morphology is similar to fibroblasts. These two cells are also very similar in their ability to produce hormone. Both have been shown to secrete estrogenic hormone.

While the embryologic, physiologic, and histologic features of these two cells are quite similar, tumors derived from them are distinctly different. In case of the theca cell tumor it is found to be solid, rubbery in consistency and in general is quite like a fibroma. On the other hand the granulosa cell tumors are large, soft, rather medullary in character and are usually not so well defined as the theca cell tumors. Histologically there is also a striking difference. In theca cell tumors the cells are fusiform, contain hyperchromatic oval nuclei and frozen sections stained with Sudan III show an accumulation of fat within the cytoplasm of the cells. The granulosa cell tumors are more epithelial in character, usually appear in more solid masses and sections show much less collagen. Fat stains show accumulation of fat, not within the tumor cells but rather in the collagen and intercellular substance.

## SUMMARY

1. The clinical, gross and microscopic characteristics of the theca cell tumor have been described.
2. The probability that many fibromas of the ovary are actually theca cell tumors is suggested.
3. There is clinical evidence that theca cell tumors secrete estrogenic hormone.

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## BLOOD DENSITY IN GUINEA PIG ANAPHYLAXIS AND IN HAY FEVER ARTIFICIALLY INDUCED\*

J. H. BLACK AND HARDY A. KEMP

*From the Department of Bacteriology and Preventive Medicine, Baylor University  
School of Medicine, Dallas, Texas*

A survey of the literature reveals many reports of investigation of the various constituents of the blood in allergic persons. Many of these were done in the absence of allergic reactions, many do not indicate whether reactions were present at the time of the investigation, while a few were done to determine differences that might be due to the reaction. In conditions such as eczema it may make no difference whether lesions are present at the time of the blood study but in hay fever, asthma, and, possibly, even urticaria it may. Even the casual observer of patients suffering from a severe attack of asthma must recognize the possibility of a large water loss from the body, yet the records do not show that this has been considered in determining the concentration of various blood constituents. Certainly, it would seem that any comparison of figures obtained during an asthmatic attack and in the period of freedom would have to be correlated with changes in blood concentration.

In addition there is abundant evidence of marked change locally and possibly generally in the vascular system during an allergic attack. Local vasodilation and edema are regular findings. Changes in plasma volume would be expected as the result of changes in the caliber and permeability of the vessels. Irrespective of loss of water from the body as a result of muscular effort there should be changes in blood concentration due to the allergic reaction itself.

In order to determine if such volume changes do occur the

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blood density was studied during an attack of artificially induced hay fever. In such an investigation it was recognized that large loss of water due to prolonged muscular activity need not be considered and that it might be possible to determine if the allergic reaction, of itself, was accompanied by blood volume change.

Because of its sensitivity the method of Barbour and Hamilton<sup>1</sup> was used. This consists of the timing of a ten cubic millimeter drop of blood as it falls thirty centimeters through a mixture of xylene and bromobenzene. Using a solution of potassium sulphate of known density as the standard the density of the blood is determined from its falling time.

One of us (J. H. B.), sensitive to ragweed pollen, while at rest and without food for six hours, and without water for two hours, introduced into his nostril sufficient ragweed pollen extract to produce within three minutes sneezing, edema of the nasal mucosa and profuse serous discharge. Blood density determinations were made by Dr. Fred T. Rogers before, immediately after the onset of sneezing, and at intervals following. From chart 1 it will be seen that there was an immediate concentration of blood from a specific gravity of 1.0561 to 1.0578. This gradually declined until at the end of thirty minutes it had returned to its original level and clinical evidence of reaction had disappeared. Pollen extract again was instilled into the nostril and a second increase in density, equal to the first, occurred at once and returned to its original level a little more rapidly than before. Since each change of 0.0010 equals 2 per cent change in water concentration it is evident that approximately 3.4 per cent of the water content of the blood left the vessels so rapidly that its passing was completed before blood could be drawn, though this was done as quickly as possible after the first sneeze occurred.

This finding we felt had a bearing not only on the subject of "blood chemistry" in allergic persons but also on the question of the mechanism of the production of the allergic reaction.

There is still much argument as to the possible identity of allergy and anaphylaxis. Concentration of the blood in anaphylactic animals has been reported<sup>2,3,4</sup> as a regular accompaniment

of the anaphylactic reaction. The similarity of vascular reaction in these two conditions might add a small bit of evidence of their identity.

It was felt that some criticism might be made of the methods which had been used in determining blood concentration in anaphylactic animals. Manwaring, Hosipean and Beattie<sup>2</sup> used dogs in their experiments and used as a criterion changes in red cell count. They concluded that the results were unsatisfactory because of "hepatic sinusoidal stasis." Simond's<sup>3</sup> hemoglobin determinations in dogs were used to indicate the amount of concentration. He found some concentration of blood in most of the animals but a decrease in a small percentage of the dogs. Since it is known that the characteristic change in anaphylactic

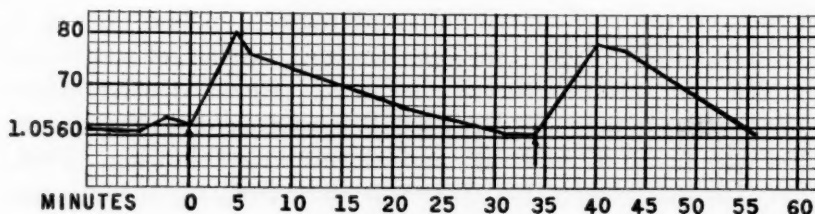


CHART 1

dogs is a marked change in the hepatic circulation this makes interpretation of red cell counts and hemoglobin determinations difficult and open to question. Drinker and Went<sup>4</sup> used guinea pigs and demonstrated by a modification of the dye method that "if asphyxia was prevented no change occurred in blood volume. When shock was exceedingly severe and some degree of asphyxia unavoidable the blood volume reading during the height of the asphyxia, was low due to poor mixing of the injected dye." However, their determinations were made with sodium barbital as an anaesthetic and with intravenous injection of curare and artificial respiration was used to prevent the development of asphyxia, all of which might materially modify the reaction of the vascular system. In the hope that we might be able to follow the blood volume change we used the method of Barbour and

Hamilton and avoided the tremendous influence of the liver in dogs by using guinea pigs in the following tests.

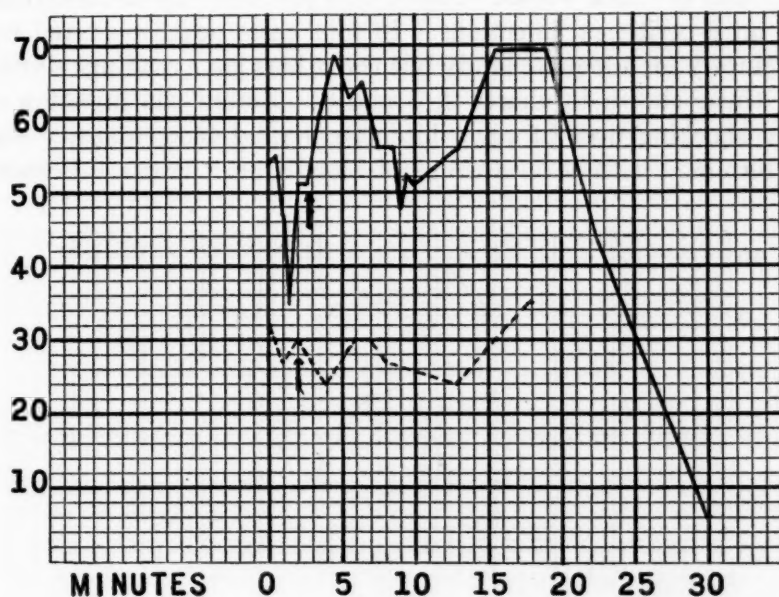


CHART 2

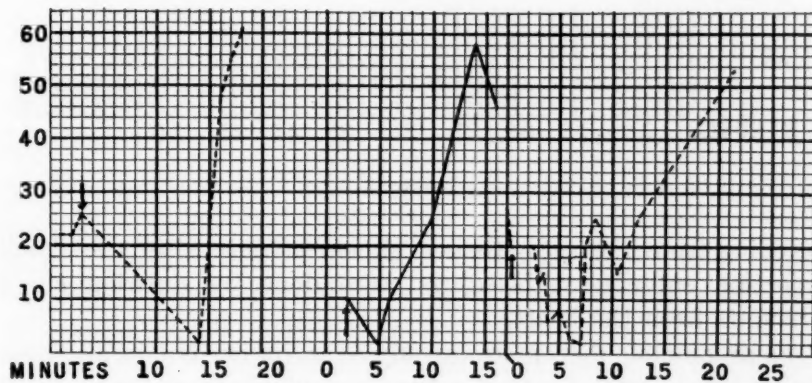


CHART 3

Eighteen guinea pigs weighing approximately three hundred grams each were injected intra-peritoneally with 1 cc. of 1 per

cent egg white. Thirteen of these were given their shock injection after two and one-half weeks while five were injected at the end of three weeks. Shock was produced by intra-cardiac or intra-peritoneal injections. In four cases death occurred too promptly for adequate study but the others gave readings similar to those shown in charts 2, 3 and 4. Before the injection of the shocking dose of egg white the normal blood density was determined with several drops of blood from the ear. Because

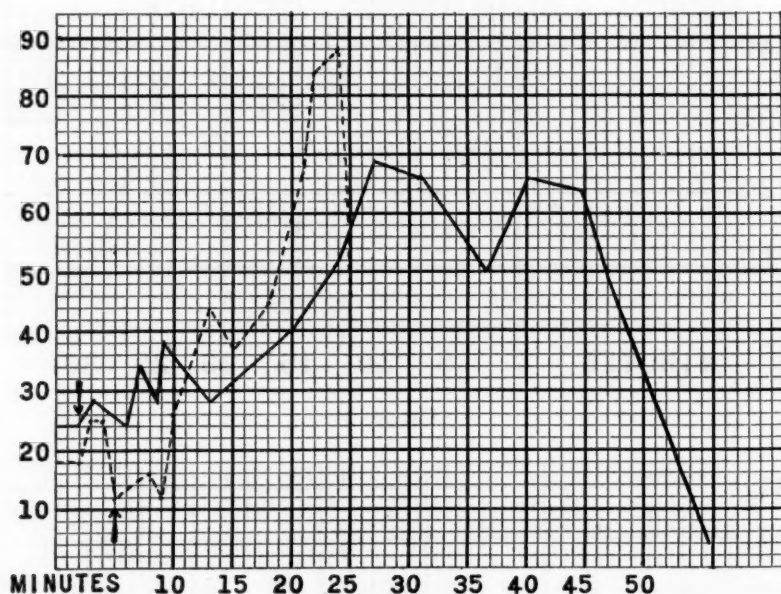


CHART 4

bleeding from the ears stopped almost at once after the shock injection subsequent bleedings were done by drawing small amounts from the heart.

Some animals died more quickly than others but in all those living long enough for even a few determinations the increase in density may be seen. Chart 2 illustrates the change in two animals who showed only a moderate degree of anaphylaxis. Chart 3 shows the change in three animals with fatal anaphylaxis

with death occurring quickly. Chart 4 is the record of two cases whose death was more delayed.

#### DISCUSSION

Barbour<sup>5</sup> stated that in his work on animals he had found the emotional reaction of the animal making a tremendous difference in the concentration of the blood. If this were true it would make the method impracticable except for animals under anaesthesia and this we wished to avoid because of its possible effect on the blood concentration. Reference to the charts shown will

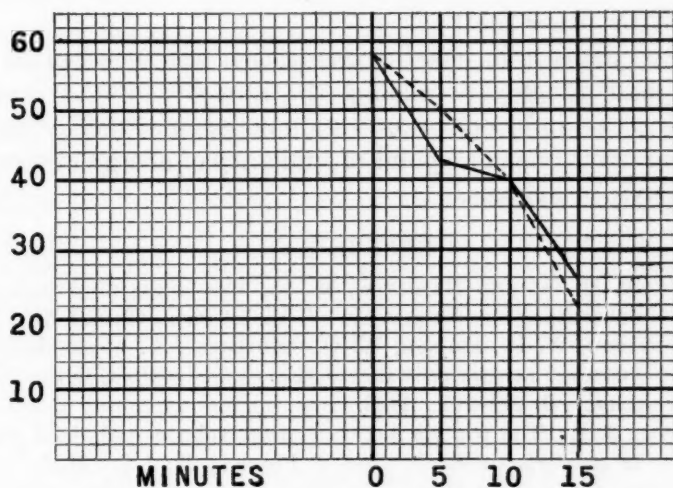


CHART 5

demonstrate that the emotional reaction in guinea pigs made no appreciable change in their blood concentration since several readings made before the shock injection show very small variations. In these animals, at least, we feel it has been demonstrated that the method is entirely practicable.

In spite of the repeated withdrawal of small amounts of blood the density of the blood increased markedly in all pigs. In contrast with these findings are those in three normal pigs as shown in chart 5. These animals, bled in the same manner as the others at five minute intervals, withdrawing the blood from

the heart show a definite decrease in density, possibly due to the influence of flow of fluid from the tissues to replace that lost by the bleeding. Since the normal trend of the curve is downward because of repeated bleedings the increase in density in the anaphylactic animal becomes more significant.

#### CONCLUSIONS

The method of Barbour and Hamilton was found to be well suited to this type of investigation since it makes possible frequent rapid determinations with very small amounts of blood and detects small changes in density which could not be so certainly detected by other methods.

Marked increase in blood concentration was found in all pigs, the increase roughly paralleling the intensity of the reaction.

The increase in blood concentration noted in artificially induced hay fever offers one more point of similarity of allergy in men and anaphylaxis in animals.

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## THE PREPARATION OF DEXTROSE SOLUTION FOR INTRAVENOUS ADMINISTRATION\*

(Continued)

WILLIAM J. ELSEY AND RALPH G. STILLMAN

*From The Central Laboratories, New York Hospital and the Department of  
Applied Pathology and Bacteriology, Cornell University Medical College*

*Storage.* Flasks are stored at room temperature, away from direct sunlight and in our experience remain suitable for use for an indefinite period. Fuqua (11) recommends that solutions be kept in low temperature refrigerators "to prevent possible fermentation." But fermentation will not take place in a sterile solution and many of the molds which we have especially to fear, grow readily at refrigerator temperature. Some authors recommend that dextrose solutions be used within 24 hours after manufacture while others state they must never be kept longer than 7 days. The solution as we make it has been introduced into the circulation of patients 21 months after preparation and the ampules found on the market may frequently have an age of several months before they are sold by the retailer. The acidity which develops in these solutions on standing does not, in our experience, favor the occurrence of reactions.

Our package was designed for use within the hospital and is not particularly well suited for shipment. It can, however, be transported safely with reasonable care. Flasks that were inverted once daily for a period of two weeks showed no growth after standing for a further period of two weeks and were injected into patients without untoward results.

*Equipment for administration: Glassware.* The particular type of apparatus used for administration will naturally vary with the operator. The glass parts will not contribute to the occurrence of reactions so long as they are properly cleaned and sterilized.

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*Rubber tubing.* Most authors have emphasized the importance of the proper preparation of rubber tubing. New tubing must be subjected to a cleansing process in order to remove the fine powder, principally sulphur, which covers it, inside and out. All traces of blood and of dextrose must be removed from used tubing before it is sterilized. Rademaker<sup>32,33</sup> states that water in which new rubber stoppers had been boiled did not cause reaction in animals and Seibert<sup>37</sup> says that an extract made by boiling corks "gave only a slight fever in proportion to the deep color" of the solution. Certain varieties of pure gum or acid-cured red rubber tubing have been used successfully without preliminary treatment but tend to disintegrate upon repeated sterilization.

More economical is the use of pure gum, heavy wall, black tubing which is somewhat more expensive but is more resistant to heat. It requires preliminary treatment.

Before use this tubing is cut into the lengths that are to be used in the apparatus. Boil 15 to 20 min. in dilute (0.5 to 1.0 per cent) sodium hydroxide, care being taken that it is completely filled with the solution. Rinse thoroughly in tap water while twisting or manipulating or scrubbing it on the inside with wads of gauze attached to a string. Soak in dilute (0.5 to 1.0 per cent) hydrochloric acid 15 to 20 minutes, care again being taken that the lumen is completely filled with the solution. Wash in a strong stream of tap water 15 minutes then in 4 or 5 changes of distilled water after which it is ready to be assembled in the apparatus. After use the apparatus is immediately taken apart and the tubing placed in soapy water or in a solution of one of the non-soap trisodium phosphate cleaning powders now generally used. Wash thoroughly in this solution, rinse vigorously in tap water and in 4 or 5 changes of distilled water and it is ready again for assembly in the apparatus. It is important that every endeavor be made to avoid the coagulation of blood in the tubing because this material is well suited for the production of a foreign protein reaction, especially after being autoclaved. This should be forcibly impressed upon those who use and care for the apparatus for it is easier to prevent the formation of a clot than to remove completely one already formed.

*Administration: Temperature of the solution.* Originally it was generally agreed that any solution introduced directly into the circulation should have the same temperature as that of the blood when it reaches the vein. Of late, however, some writers have denied the necessity for this. Banks<sup>1</sup> used temperatures from 68° to 113°F. safely in his animals and Darrow<sup>4</sup> states that temperatures between 20° and 42°C. are permissible. Little<sup>25</sup> maintains that a cold fluid is safe while a hot one is dangerous as it is likely to liberate fibrin and produce emboli. Walter<sup>44</sup> claims he has given solutions at from 20° to 44°C. without reaction. Nevertheless, common sense dictates that the solution should have the temperature of the blood and from our experience we have concluded that the use of a cool solution may at times be detrimental. The only reaction we have seen after an infusion of salt solution alone followed the introduction of a cool solution and more than once when investigating a reac-

tion, we have been told either that the solution was cool or that the operator was not sure it was warm. We lack positive information about the temperature of hundreds of infusions that have been given without subsequent reaction, but urge that in all instances fluids be introduced into the circulation when at body temperature.

*Rate of flow.* Many writers from Levy<sup>24</sup> to the present time have called attention to the danger of "speed shock" and the tendency is to recommend that the rate of injection of most fluids be limited to 3 to 5 cc. per minute. In contrast Caughey<sup>3</sup> describes the injection of 1500 cc. of normal saline in 30 minutes to more than 25 patients without reaction and Milbert<sup>29</sup>, in a series of 25 cases injected several kinds of solutions at rates varying from 61 to 177 cc. per minute and saw chills following in three instances, two occurring more than an hour later being described as slight. Milbert quotes Unger as saying he had never seen speed shock from too rapid transfusion of blood. The incidence of reactions in Milbert's series (12 per cent) is much higher than we have experienced with our solution and higher than should ever occur. Seibert<sup>30</sup> maintained that in her experimental animals the reactions occurred entirely independently of the rate of administration. Recently Hyman and Hirschfeld<sup>17</sup> have re-emphasized the necessity of a slow rate of injection and claim that even an antigen may be injected intravenously into a sensitized animal without shock, provided it is done slowly enough.

Theoretically the speed with which a fluid can be safely introduced into the circulation should depend upon the nature of the fluid; that is those fluids most closely resembling the blood can be introduced more rapidly than those which differ greatly, especially in tonicity. This is the reason why speed shock rarely follows rapid injection of whole blood. Hypertonic solutions must be injected more slowly and 50 per cent dextrose with great deliberation. Few house officers realize that this latter solution readily produces phlebitis and thrombosis in a vein, that in fact it has been used to cause occlusion of varicose veins, and must, therefore, be injected so slowly that the intima of the vessel is at no time exposed to the full strength of the solution. The total volume of the fluid is also important since small volumes may be injected more rapidly than large. With large volumes the danger of overtaxing the heart must be kept in mind.

From our experience we are convinced that the percentage of reactions will be larger in cases in which rapid injection is practised. We have often found the only explanation for the occurrence of a reaction was a rapid rate of injection. Therefore, we recommend that the following rates be regarded as the upper limits of speed permissible in intravenous infusions:

Blood (transfusion).....	25-50 cc. per minute
Physiological saline. ....	10-20 cc. per minute
5 per cent dextrose.....	5-10 cc. per minute
Hypertonic sodium chloride.....	2- 5 cc. per minute
50 per cent dextrose.....	1- 2 cc. per minute

The rate of injection should be uniform. Hendon<sup>16</sup> states he has been able to produce chills by "milking" the rubber tubing during the injection. He ascribes the effect to the interruption of the flow but it may have been due to dislodging something attached to the inner surface of the tubing. Irregularity of rate may occur when the operator has difficulty with his technic as when the needle becomes obstructed, or escapes from the vein.

In phleboclysis or intravenous "drip" the rate should always be very slow<sup>8</sup> but it is not unusual for a chill to occur during the first hour. Matas<sup>28</sup> regards this as of little importance and the patient may seem to suffer no permanent harm. Nevertheless, when Jeschek<sup>19</sup> made a histologic study of the veins in 10 cases he found the vein thrombosed in four, various injuries to the vessel wall in four and in only two was the vessel completely unchanged. He states that in 200 patients there was no death from embolus after continuous drip infusions but in 16 it was possible to demonstrate emboli in the smaller pulmonary arteries and in three of these the origin was in the arm vein. Friedrich and Bucholy<sup>10</sup> report a fatal pulmonary embolism following this treatment. They claim they found thrombosis in the arm vein in about one-third of the cases. Since injury to the wall of the vein readily results in setting free fibrin with a resultant chill much depends upon the skill of the operator in placing the cannula and keeping it in position. We condemn the too-frequent practice by which the house officer, after introducing the needle and starting the flow of fluid, departs and leaves the patient to the care of a nurse who may have to look after the needs of other patients at the same time.

*Susceptibility of the patient.* Matas<sup>28</sup> is unable to explain why reactions occur in only a small percentage of patients when the same solution, technic and apparatus are used unless it is a matter of individual susceptibility. Too frequent resort to this explanation is a dangerous practice but there do appear to occur changes in the condition of a patient which influence the appearance of reactions. The following illustrates such an incident.

Pt. S. H. Hyperthyroidism. Removal of right lobe and isthmus of thyroid in 1916. Recurrence of symptoms. Operation for removal of portion of remaining thyroid. Sequence of events as follows:

February 20, 1931	9:00 A.M.	Sodium amytal, gr. iii
	10:45 A.M.-12:00 noon	In operating room
	12:15 P.M.	Conscious
	2:45 P.M.	100 cc. 50 per cent dextrose in 5 minutes. Lot AY. No reaction.
	8:15 P.M.	50 cc. 50 per cent dextrose in 5 to 6 minutes. Lot AY.
	9:20 P.M.	Chill lasting 12 minutes.
	10:15 P.M.	Pantopon, ampule i.
February 21	12:05 P.M.	Pantopon, ampule ss.
	5:00 P.M.	50 cc. 50 per cent dextrose in 5 to 6 minutes. Lot AZ.





tion of a number of expedients in various places is reported to have been followed by excellent results. In this method, however, the dextrose, a relatively unstable substance, undergoes less change than that which necessarily occurs in all other methods that have been proposed. This method is inexpensive and expeditious and can be followed by any careful person who is experienced in bacteriologic technic.

*Method.* When a large quantity of the solution is prepared it is convenient to extend the procedure over two days. In this way the glassware cools after sterilization and preliminary work is done on the first day while the second is devoted to actual filtration and packaging of the solution. When smaller quantities are prepared all of this work can be done on one day.

*Preparation of containers.* The containers are 125 cc. Pyrex Florence flasks which are used for no other purpose than 50 per cent dextrose. A system of exchange whereby the clinical units obtain a full flask only in return for an empty one has prevented their loss or disappearance. Upon return to the laboratory they are washed in running hot tap water, inverted for drainage and drying and set aside until needed. Those not clean after this are treated with chromic acid cleaning solution. On the first day the mouths of these flasks are covered with loosely fitting aluminum caps\* of about the same length as the neck (glass shell vials have also been used), the flasks are arranged in wire baskets and autoclaved at 18 pounds for 30 minutes. These caps remain until replaced by rubber stoppers when the flask is filled. Their efficiency has been demonstrated in their use to cover flasks containing sterile broth. Such flasks left in the open room have remained sterile for at least one month. Their use does away with the employment of cotton plugs which have a tendency to drop fibres into the flask.

*Preparation of apparatus (fig. 1).* The filter-flasks are 2-liter round bottom, ring-neck Pyrex flasks provided with an outlet of glass tubing at the bottom and a longer piece of tubing containing a small bulb fused into the side of the neck. This bulb is filled with non-absorbent cotton which acts as an air filter. The tube at the bottom is connected to a hooded tip (sterile filter) with rubber tubing to which a pinch-cock is fitted. The mouth of the flask and the opening of the sterile filter are covered with cellophane and the opening of the side tube in the neck plugged with cotton. The flasks are then autoclaved at 18 pounds for 30 minutes. In use these flasks rest upon rings which are supported by rods firmly fixed to the top of the bench.

The connection between the filter-flasks and the glass manifold attached to the Berkefeld filters consists of a rubber stopper which fits the mouth of the flask and through which passes a glass tube which is connected to 3-way Pyrex glass stopcocks with heavy-wall (compression) rubber tubing. Three filter-flasks require three rubber stoppers and two stopcocks as shown in the diagram. The rubber stoppers are wrapped in cellophane and the free end of the rubber

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\* Obtained from John M. Marris Co., 52 Walker St., New York City.



tubing is covered with a test tube and the whole autoclaved at 18 pounds for 30 minutes.

Berkefeld filter candles (No. 1 N) of which we use five, are connected with compression tubing to a glass manifold. The open end of the manifold is covered with a glass test tube. The filter candles are wet with distilled water and together with the manifold are autoclaved at 10 pounds for one hour.

The suction line is fitted with "T" tubes to which there are attached by compression tubing 3-way Pyrex glass stopcocks to the side arm of which there is attached a piece of glass tubing in which a bulb has been blown. This bulb is packed rather tightly with non-absorbent cotton to act as a "brake" on the

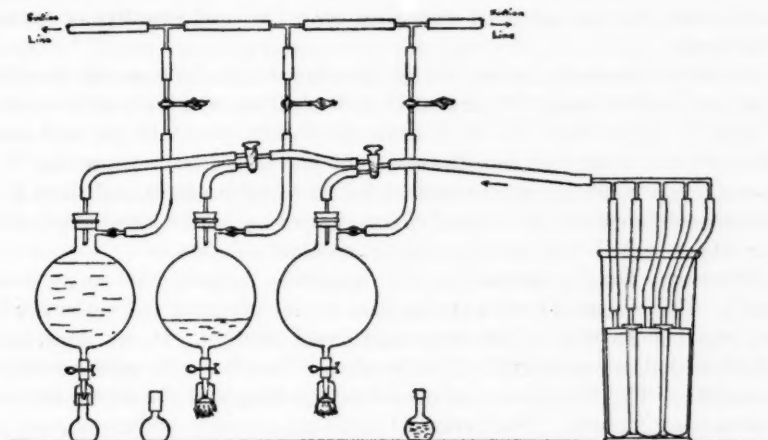


FIGURE 1. APPARATUS FOR FILTERING DEXTROSE SOLUTION

The glass beads in the "arm-jar" at the right and the rings and rods that support the filter flasks at the left have not been included so as to avoid confusing the diagram.

air which enters when the suction is cut off. Compression tubing is attached to the other arm for connection with the filter flasks.

For holding the Berkefeld filters we have used what is called an "arm jar" which is slightly wider at the top than at the bottom.

By the substitution of cellophane and aluminum caps for the commonly used cotton plugs, an important source of contamination with foreign bodies, so-called "fish," is excluded. It may be added that in spite of all precautions these "fish" occasionally appear in the solution. We believe they probably occur because it is impossible to insure the removal of all foreign bodies from all of the containers used, by the practical methods of cleaning we are forced to adopt.

Preparation of solution. The solution also is prepared on the first day.

Freshly distilled water is collected in 5-gallon glass demijohns which are kept inverted when not in use or in a large Pyrex glass reservoir which is drained daily. Eighteen liters of this water is heated to 50° to 60°C. and placed in a white enamelware stock-pot of about 30 liters capacity. A mechanical stirrer is placed in the water and operated while 12 kgm. of U. S. P. dextrose or 10.8 kgm. anhydrous dextrose is added slowly. Solution is complete in a few minutes and the temperature of the solution approximates room temperature. The stirrer is removed and the volume made up to 24 liters with freshly distilled water. The volume may be indicated by a mark on the side of the pot or a gauge made for the purpose. The solution is mixed with a glass rod, the pot covered and set aside in the refrigerator until the following day. Two, three or four of these pots are prepared depending upon the total quantity of solution to be made.

Authorities generally ignore the fact that 50 per cent dextrose may be either 50 per cent anhydrous or 50 per cent U. S. P. dextrose and both solutions may be found in the market. U. S. P. dextrose contains about 10 per cent water and therefore 10 per cent less dextrose than the anhydrous. Since the U. S. Pharmacopeia is the accepted standard for therapeutic agents and there is no discernible difference in the clinical effects of the two solutions we have decided upon 50 per cent U. S. P. dextrose as our standard solution.

Filtration. On the second day the apparatus is assembled in a "sterile room". This is a small room that has been made dust-proof and before use has been wiped down with dilute compound cresol solution. It can be supplied with filtered air when desired. The apparatus is handled with care to maintain its sterility. The filter-flasks are placed on the rings and the rubber stoppers fitted to their mouths. The Berkefeld filters are placed in the arm-jar and the space about them filled with glass beads so as to reduce the amount of solution required to bring its level above the filtering surface. The open end of the manifold is connected with the rubber tubing leading to the filter flasks. The tubing from the suction line is now connected to the side tubes in the necks of the filter flasks, the arm-jar filled with dextrose solution and the apparatus is ready for operation. The arrangement of the stopcocks is such as to enable the operator to apply suction to any of the filter-flasks and to release it at will and to permit the filtered solution to run into any one of the flasks. A little practice enables the operator to manipulate the stopcocks without error. They should always be opened and closed slowly to insure gradual changes in pressure.

While one filter-flask is filling, one already filled is being emptied into the small flasks. Each 125 cc. flask receives 100 cc. of solution as compared with a flask containing a measured amount of water. Meanwhile No. 5 "No-Air" or similar type rubber stoppers have been sterilized by boiling in distilled water for 15 minutes. They are placed in position on the flasks after the removal of the aluminum caps. The process of filling and closing these flasks is carried out with sterile precautions.

The rate of filtration varies depending upon the strength of the suction

but averages about 15 liters per hour. Two operators can filter solution and fill and cap from 900 to 1,000 flasks in a day. Sample flasks, 15 in all, are removed from the beginning, the middle and the end of each lot for sterility tests for both aerobic and anaerobic bacteria. While we have never encountered such contamination we regard the tests as necessary for the detection of defects in the filters. On one occasion such a defect developed during sterilization after the usual method of testing the filter had been carried out. After being filled and capped, the flasks are stored at room temperature for 2 weeks when they are inspected by strong artificial light for the presence of floating bodies or contamination with yeasts or molds. Similar inspection is also made before they are issued to any of the clinical units. Flasks that show floating bodies are set aside and their contents refiltered when the next lot of solution is manufactured. When ready for issue, there is attached to each flask a tag stamped with the date and a serial number which serve for identification. When the contents of the flask are used this serial number is entered on the chart of the patient.

After completion of the work, the filter candles are immersed in tap water in which they are allowed to stand overnight. The following day tap water is drawn through the filters for 2 hours or more. The outer surface is then scrubbed with a small brush to remove the film of material that has been filtered out of the solution. Now at least 2 liters of distilled water is drawn through the filters, each one being inverted 4 or 5 times during the process in order to empty it.

This rather elaborate procedure was developed in an attempt to remove from the filters all traces of dextrose. Dextrose remaining in the filter will become caramelized when the candle is next sterilized and will impart a yellow color to the first part of the solution passing through the filter. With the above method of cleaning, the first portion of the filtered solution has only a faint yellow color. While it is probably unnecessary, we are in the habit of discarding the first liter of the filtrate.

Before sterilization, the filter should be tested for cracks or other imperfections by immersing it in water, connecting it to a source of compressed air and observing the bubbles that rise from it. These should rise evenly, a crack being revealed by the escape of larger bubbles along a line or in one locality. Care must be observed to avoid the application of too high pressure or a crack in the filter may result.

The tubing, both rubber and glass, is washed thoroughly in hot water, allowed to dry and stored under protection from dust until needed. The "No-Air" or similar stoppers when new are washed thoroughly in hot water and soap and rinsed in distilled water. After use, they are washed in hot water only. They are used repeatedly until so stretched they no longer act as safe stoppers. The rubber tubing is pure gum, thick wall, so-called "pressure" tubing and will withstand repeated sterilization.

By this method, allowing for materials, including replacement of broken

glassware, worn out rubber and other apparatus and the purchase of dextrose, but not including labor or overhead, the cost of the solution is not more than 2 cents per 100 cc. The cost of installation of the necessary apparatus is in the neighborhood of \$175.00.

This method has been in use at the New York Hospital since April 1, 1928. From that time until January 1, 1936 there have been issued approximately 33,000 flasks. During this period every endeavor has been made to have all reactions reported to the laboratory and each one reported has been investigated in an attempt to discover the cause. The record for the year 1935 is given in detail since it is representative of the entire period. 9,140 flasks were issued representing 13 lots of solution of which approximately 95 per cent were injected intravenously. Of these 8,683 infusions, 13 were followed by reactions of chill and rise in temperature. This is an incidence of 1:668 or not quite 0.15 per cent. When the records of these reactions were examined, no instance was found in which there was reason to assume that the glucose solution as such was the cause. They were distributed throughout the year one for each lot of from 600 to 1,000 flasks. Each patient received either 5 per cent or 10 per cent solution. These reactions presented the following characteristics:

Case 1. Female. Patient was running a septic temperature and having chills. The occurrence of a chill following the infusion may have been coincidental. Received another infusion without reaction.

Case 2. Female. Patient was running a septic temperature and having chills. The occurrence of a chill following the infusion may have been coincidental. Received other infusions without reaction.

Case 3. Male. It was reported that there was difficulty in the administration. The needle apparently became obstructed and an unusual amount of manipulation was resorted to before the operation was completed. Received other infusions without reaction.

Case 4. Female. Patient's veins were small and it is reported that several attempts were made before the infusion was running smoothly.

Case 5. Female. Patient was suffering from hyperthyroidism and after thyroidectomy received 1,000 cc. of solution at the rate of 40 cc. per minute. Received other infusions without reaction.

Case 6. Female. Patient received 1,000 cc. of solution at the rate of 30-35 cc. per minute. Received other infusions without reaction.

Case 7. Female. Patient had just been subjected to prolonged and difficult operation on stomach. Operating surgeon regarded chill as "part of the post-operative reaction."

Case 8. Female. Reaction occurred 2 hrs. after infusion and patient's temperature had begun to rise some hours before. Received other infusions without reaction.

Case 9. Male. Patient was suffering from advanced carcinoma of the bladder and was in desperate condition. Received 1,000 cc. of solution at the rate of 16 cc. per minute.

Case 10. Female. Condition of patient was serious. Received other infusions without reaction.

Case 11. Female. Patient was running an irregular temperature. Received 900 cc. at the rate of 20 cc. per minute.

Case 12. Female. Patient received 600 cc. at the rate of 13 cc. per minute. Received other infusions without reaction.

Case 13. Female. Patient received 900 cc. at the rate of 15 cc. per minute.

In 8 of these 13 cases the patient received one or more other infusions frequently from the same lot of solution of dextrose without a reaction. These reactions were scattered throughout the year and in most instances only a single reaction occurred with one lot of solution. This suggests the possibility of variations in the susceptibility of the patient or in the success of administration. Eleven of the patients were females. The significance of this figure is not clear for the hospital includes a large obstetrical and gynecological service without any corresponding unit restricted to males, so it is likely the number of females receiving infusions is greater than males so treated. The preponderance is so great, however, as to suggest that the female is more susceptible to reactions than the male.

Although in our experience no reaction has proved fatal we cannot wholly subscribe to Matas' opinion<sup>28</sup> that it should be "regarded as an epiphenomenon which, while disturbing and tempestuous, is not per se of grave significance." The violence of the chill shows a radical disturbance in body processes and the absence of fatalities does not mean a lack of importance. Lee<sup>23</sup> shows that temporary changes occur in the nuclei of some of the cells of the central nervous system following the injection of hypertonic solutions in animals and other changes may be present. Nor do we agree with Hanzlik and Karsner<sup>14</sup> who claim that in practically all instances intravenous infusion results in definite damage to the animal (guinea pig). Their work is open to criticism. They state they made their injections slowly and the doses were those used in therapeutic practice. But when



translated into corresponding quantities per gram for an animal weighing 70 kgm., they involve the injection at rates of 400 to 1300 cc. per minute of such doses as 1129 cc. 1 per cent collargol, 700 cc. of dog bile, 1050 cc. typhoid bacterin, 1093 cc. agar, etc. Although they apparently believe intravenous injections "distinctly harmful" they content themselves with stating "intravenous administration of agents which still lack a scientific basis is unjustified" and warning "against promiscuous, careless and unwarranted use of the intravenous method." The publication of such conclusions may well have had a deterrent influence on the use of the intravenous method which would not have been the case had their data been properly translated into values such as are employed in the human. Thousands of intravenous injections are being given to patients daily with evidence of only beneficial effect. On the other hand, reactions are alarming to the patient, his family and friends and, even though they may not cause permanent damage, every possible effort should be made to reduce them to a minimum.

The limitation of our output to 50 per cent dextrose was made for several reasons. The issuance of a single product greatly simplifies the problem of manufacture. The demonstrated bactericidal powers of the 50 per cent solution enable it to be stored and handled with confidence in its sterility. The solution is concentrated so the quantity made up at one time provides a supply for a longer period and the small package can be handled easily and stored compactly. The necessity for dilution at the place of administration has not introduced danger of contamination nor appreciably complicated the operation. The issue of a single concentrated solution permits free choice of diluent by the clinician. Some physicians believe that reactions are fewer when the diluent is distilled water, while others think salt solution is safer. Dinsmore and Buerki<sup>6</sup> state that the addition of dextrose to Ringer's solution "almost inevitably" causes reactions. Our experience with this diluent is limited but we have not seen reactions following its use. Five per cent dextrose in distilled water is nearly isotonic but in saline the solution is hypertonic. At the New York Hospital sterile freshly distilled water and



sterile saline can be obtained in one-liter flasks. The diluent is selected as desired, the proper amount is poured away and one, two or three flasks of 50 per cent dextrose added, giving 5, 10 or 15 per cent dextrose as desired. In our experience the nature of the diluent does not appear to have any influence in the production of reactions.

The method may be adapted to the manufacture of practically any solution intended for intravenous use even when heat sterilization appears desirable. When the use of heat must be avoided the filtration method of sterilization becomes almost indispensable. We have used modifications of the method in the preparation of buffered 2 per cent sodium citrate, 2½ per cent sodium citrate for transfusions, concentrated Ringer's solution and concentrated sodium lactate (Hartman) and liver extract (Castle) for intramuscular injection. The substitution of the Seitz, Mandier or Chamberlain filter does not alter the principle of the procedure.

#### SUMMARY

Examination into the reputed causes of reactions following intravenous injection of dextrose solution reveals that some are more important than others.

1. The principal factor has been the use of water which has stood for some time after distillation and before sterilization. This is corrected by the use of only freshly distilled water produced in a properly designed and operated still. Double or triple distillation is not essential.

2. The presence of particulate matter in the solution can be prevented by proper filtration and avoidance of cotton plugs or fabric covers for flasks and other containers.

3. Reactions due to the rubber tubing may be avoided by following the procedure outlined. Thorough cleaning is especially important in the preparation of new tubing.

4. Too rapid or irregular introduction of the solution into the circulation or unskillful performance of the operation is to be avoided by care in technic. A table of recommended speeds for injection of several solutions is given.

5. The susceptibility of the patient may be of importance and cannot be predicted but there is some evidence that the administration of a sedative may prevent the development of a chill. Hyperthyroidism is possibly a predisposing cause.

6. In all intravenous infusions the solution should reach the vein at a temperature closely approximating body temperature.

7. There is described a method for the preparation of a 50 per cent solution of U. S. P. dextrose that is simple and inexpensive and effective in the production of a solution that is clear, colorless and sterile and the use of which does not produce reactions. It is issued to the clinical services and is diluted to the desired strength at the place of administration in the diluent selected. This solution remains usable for an indefinite period and changes in pH which take place in it are not of importance. It has definite bactericidal powers which contribute to the safety of its use. The method has been in use for eight years during which time 33,000 100-cc. flasks have been issued. In one year reported in detail the incidence of reactions was 0.15 per cent. The essential features of the method are the accomplishment of clarification and sterilization of the solution in one operation by filtration through a bacteria-proof filter (Berkefeld). The dextrose is at no time subjected to the action of heat which brings about changes in this material.

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## PART II

### ABSTRACTS OF PUBLICATIONS ON NECROPSIES, 1931 TO 1934 (Continued)

Prepared by the Committee on Necropsies of The American Society of Clinical Pathologists for the year 1934-1935.

I. DAVIDSOHN, M.D., *Chairman.*

A. HELWIG, M.D.

O. SAPHIR, M.D.

M. WARWICK, M.D.

*The clinical pathologist as a teacher.* Editorial. Jour. Am. Med. Ass. 101: 2124, 1933. The scientific work of hospital revolves around the laboratory. Intelligent correlation and interpretation of information gotten from necropsies will create interest in morbid anatomy and give a real incentive to obtain permission for more and more necropsies. The exercise of restraint on the part of the pathologist will accomplish much more than a practise of forcing his opinions and decisions on his fellow practitioners.—M. Warwick.

*Letter.* Joseph Doane, former superintendent of Philadelphia General Hospital. Jour. Am. Med. Ass. 101: 873, 1933. Autopsies can be obtained by anyone with the proper interest and information of proper methods. At his hospital they have a weekly conference on the deaths and the necropsy record of each interne. Clinicians should take part in this and not leave it to the pathologist.—M. Warwick.

*Exploring death.* H. I. Corper. Am. Jour. Clin. Path. 3: 145, 1933. This is a philosophy of inevitable death and a history of necropsy work.—M. Warwick.

*The present status and future development of legal medicine in United States.* O. T. Schultz. Arch. Path. 15: 542, 1933. The present unsatisfactory status of legal medicine in the United States is the result of the American system of criminal judicial administration, in which the basic medicolegal element is the coroner. This office functions poorly because its duties and authority are not clearly defined by law, it is an obscure elective office and the incumbent need have no especial fitness for it. It was transplanted from England, it is not adopted to present conditions and is archaic. Usually there are provided no facilities for scientific work. The office of medical examiner works much better, but it, too, is not well enough financially supported to use scientific meth-



ods in its work. Also in most jurisdictions, unbiased psychiatric opinion is not fully and freely available in the agencies of justice. Psychiatry has not received the legal recognition which it deserves. Also the present contentious method of presenting expert medical testimony has hampered the development of legal medicine. The future development of legal medicine will take place through agencies such as medical institutes functioning through the office of coroner or medical examiner, and agencies which will make impartial psychiatric opinion available to courts and other agencies of justice. These should be under state university in many states. The development of legal medicine as a science of practical application should go hand in hand with the development of forensic medicine as a university discipline.—*M. Warwick.*

*Findings of special clinical interest revealed at postmortem examination.* W. I. Deadman. *Can. Med. Ass. Jour.* **28**: 401, 1933. This is a description of numerous unexpected pathological conditions disclosed at necropsy. Many of them were cases of sudden death.—*M. Warwick.*

*How to obtain 94.2 per cent of autopsies.* I. R. Smiley. *Mod. Hosp.* **36**: 89, 1933. (Oct.) This is the best record of any general hospital in the United States. Much depends on the pathologist. He teaches the new internes the importance of necropsies and how to approach the relatives and ask for permissions for them. He checks up on all very ill patients and sees that the way is paved for the request for a necropsy. He performs the necropsy within an hour after death, either day or night. Also he will go out around the city and do a necropsy for any member of the staff at any time. All internes are called for every necropsy and the pathologist makes of it a pathological demonstration. If it seems necessary, members of the staff will come to the hospital at any time, day or night, and ask for permission themselves.—*M. Warwick.*

*The pathological conference should be an open forum.* G. Daledorf and C. H. Munger. *Mod. Hosp.* **40**: 61, 1933 (June). The necropsy is very important but fails in its purpose if it doesn't teach its lesson to the clinicians. So pathological conferences should be held regularly but not too often. They must be very informal and not at all "high hat" but the material for them must be carefully prepared and the proceedings mimeographed. Specimens from surgical pathology should be included in it. A short clinical history should be given first and the pathological material should be shown well and be well prepared so that it is not at all objectionable. The pathologist must have a fair, unbiased attitude toward clinical errors.—*M. Warwick.*

*Relation and value of postmortem examinations to the welfare of the United States Army.* H. E. Robertson. *Mil. Surg.* **72**: 319, 1933. Offensive and defensive measures against disease are very important in the army and must be carried on in time of peace. One of the most effective measures for studying the effects and causes of disease is the examination of the bodies of those who have died from any cause. Necropsies are done not only to determine why the patient dies, but also what happened to him while he lived. In the American Expeditionary Force, a general order for necropsies on all men or officers who



died was given. This is the first time in history that such an advance was made. In war-time much can be learned such as paths of missiles which penetrate the body, differentiation of causes of shock, sites and causes of fatal hemorrhage, control and course of gas gangrene, possibilities of surgical repair of nerves, blood vessels and organs, the incidence of tuberculosis, results of poison gases, early detection of epidemic diseases, collection of data in regard to venereal disease, accurate estimation of degenerative conditions present in army can be shown by necropsies. In peace times, necropsies are necessary to show the effects of epidemics, statistical data on the development of organs in peace-time forces, the effects of various kinds of exercise, special rations and exposure to untoward climatic influences, to help train new pathologists, organize compact equipment suitable for transport and field hospitals and to educate both officers and men of both line and medical forces in the value of information obtained by this procedure. Necropsies on veterans of wars will show after effects of wounds and disease, the efficiency of medical care during life and the exact degree of physical disability produced by the exigencies of active army life. These data form the base for future methods of care, rating for disabilities and reconstruction activities. And also, the necropsy record fills out the complete medical history as related to army experiences.—*M. Warwick.*

*Why should postmortem examinations be done?* M. Warwick. *Hygeia*. 11: 606, 1933. This is an article written for the laity and considers the benefit of a necropsy to the family, the attending physicians and to the development of medical science. The family will learn of familial diseases, be better able to collect insurance and will have the satisfaction of knowing just what disease processes were present at the time of death. The attending physicians will learn, gain knowledge and experience and be better able to treat others. New and unusual diseases will be recognized and results of treatment checked. Isolated, unusual cases will be presented at medical meetings and published in medical literature where they will be a source of benefit to those who come later.—*M. Warwick.*

*The evolution of the modern autopsy table.* M. Warwick. *Woman's Med. Jour.* 40: 253, 1933. Necropsy table consisting of a large, shallow pan covered by movable slabs was first used by Robertson. Various modifications of that table have been made. These are briefly described by Warwick who presents her own version of monel metal as the ideal table.—*M. Warwick.*

*Necropsy performance in approved hospitals.* Report of Medical Education and Hospitals of the American Medical Association. 101: 690, 1933.

Percentage	1926	1928	1930	1931	1932
Over 70.....	14	16	19	24	20
50-70.....	21	45	56	62	71
30-50.....	68	132	164	210	214
15-30.....	146	249	354	295	316
Under 15.....	329	189	71	73	62

*Major Daniel O. Livery left body to science.* New York Times. Dec. 2, 1933. Major Livery, director of China Famine Relief, died at home, but bequeathed his body to the New York Post Graduate Hospital for necropsy and directed that the hospital make whatever provisions it deemed necessary in disposing of his body to provide an incentive for others to make similar provisions in their wills.—*M. Warwick.*

*Editorial. I. Marcus.* Chicago Daily Jewish Courier. Dec. 31, 1933. The editor visited the pathological laboratory of Mount Sinai Hospital, Chicago and described what benefits may come to mankind through postmortem examinations with the filing of records at the hospital. He quotes Rabbi Levinthal as saying "if any other sick person benefits from a postmortem examination, such an act is a consecration and not a desecration of the dead body."—*M. Warwick.*

*The late Calvin Coolidge and an autopsy.* Coupal. Time, Jan. 1933 and New York Times, Jan. 6, 1935. Dr. James Coupal, White House Physician for President Coolidge, urged the family to have a necropsy on the late president saying that they would "be doing a great service in stimulating public demand for such postmortem determination of the exact cause of death" in the New York Times. In "Time" he said that President Coolidge in death would be a great help to medicine and a good example to the nation if a necropsy was performed.—*M. Warwick.*

*The pathology of sudden death.* Foreign letter from London Abst. J. A. M. A. 100: 788, 1933. Mentions a paper by Beford in Jour. of Path. 101: 788, 1933. Reviews postmortem records of Leed General Infirmary for 21 years, on patients dead on arrival, from causes other than trauma or suicide.—*M. Warwick.*

*Restoration of autopsied bodies—A better method.* H. W. Williams, and O. G. Henderson. New England J. Med. 211: 371, 1934. Williams recommends the following technique to facilitate embalming: The ascending aorta is cut just above the aortic valve and tied, leaving the vessel intact. A section measuring about 4 cm. is removed from the abdominal aorta just above its bifurcation. One glass tube is inserted into the upper segment another into the lower segment. Both are tied into the aorta and each is connected by a rubber tube with the external surface of the abdominal wall. If the arch and ascending aorta are removed, a glass tube is inserted into the innominate artery. The arms of a Y-shaped tube are inserted into the left common carotid and left subclavian arteries respectively. Rubber tubing connected with the stem of the Y tube and with the innominate tube, make the great vessels accessible. The rectum and vagina are tied. The internal carotids are tied in the carotid sinus at the base of the skull. The latter is filled with plaster of Paris. The calvarium is replaced and taped.—*O. Saphir.*

*Autopsy performance.* Approved internship hospitals with highest necropsy percentage. 1933. J. A. M. A. 103: 580, 1934. St. Luke's hospital Kansas City, Mo. 86.8 per cent Mount Sinai Hospital, Philadelphia, Penna., 73.2 per cent. The publication of figures showing the percentage of necropsies performed should be most helpful to prospective internes for it is well known

that an alert and efficient staff will maintain a creditable postmortem rate.—*A. Hellwig.*

*Necropsy gloves.* S. Saltykow. Wien. Med. Wochenschr. **84**: 43, 1934. It is recommended to cover the rubber gloves with sterilized white cotton gloves.—*A. Hellwig.*

*A new knife for cutting the brain.* B. Ostertag. Cbl. f. allg. Path. u. path. Anat. **61**: 50, 1934. The knife is 28 cm. long. One side is plane, the other biconcave. The brain is held in a metal frame, measuring 13 x 18 cm. The use of both instruments allows the operator to cut perfectly plane-parallel sections and replaces the expensive brain macrotome.—*A. Hellwig.*

*Ammonium sulfide in the necropsy room.* R. Beneke. Cbl. f. allg. Path. u. path. Anat. **60-81**, 1934. A bottle of Ammonium sulfide should be present in the necropsy room. By its use, old hemorrhages are easily recognized. The reagent stains blood pigment black.—*A. Hellwig.*

*Studies on rigor mortis.* O. Berner. Deutsche. Ztschr. f. gerichtl. Med. **23**: 3, 1934. Rigor mortis of the neck relaxes easily. If it is present, the conclusion can be drawn, that the body has not been moved after rigor mortis has set in.—*A. Hellwig.*

*Sudden death at work.* D. H. Kitchin. The medicolegal and criminological review. **2**: 195, 1934. Standpoint of the legal profession. Many case histories illustrating complications. When a workman dies at his work, and the work can be shown to have contributed in a material degree his dependents are entitled to compensation. The question whether the work contributed is nearly always answered on the medical evidence. Because of the limitations of medical science, the answer must often be largely a matter of conjecture. In many cases, compensation has been awarded even when the man was in such a precarious state of health that he might have died in any circumstances.—*A. Hellwig.*

*The rôle of the pathologist in the cancer problem.* G. Foord. Am. J. Clin. Path. **4**: 321, 1934. In case of death, a necropsy should be performed on all cancer patients. Particularly important are postmortem studies of cancer patients treated by x-ray or radium. Without such studies we can never know what is being done by these agents.—*A. Hellwig.*

*The medicolegal autopsy. Introduction.* F. E. Sondern. Am. Jour. Clin. Path. **4**: 1, 1934. The importance of the medicolegal necropsy and the special training needed is stressed. There is now a course of training for men wishing to follow this work, given at Bellevue. Mention is made of the Joint Autopsy Committee in New York consisting of a committee from New York Academy of Medicine, the New York Pathological Society and the Metropolitan Funeral Director's Association. It continues to function as a grievance committee.—*M. Warwick.*

*The medicolegal system of the United States.* O. T. Schultz. Am. Jour. Clin. Path. **4**: 7, 1934. The medicolegal necropsy work should be done as a government service. The laws governing dead bodies and necropsies vary in

different states. Coroners can insist on necropsies and perform them without permits only in cases where death is due to violence, otherwise, there must be a written permit before any autopsy can be done. It has long been the custom of pathologists to retain some or all of the organs, but unless this is included in the permit, their right may be questioned.—*M. Warwick.*

*The medicolegal autopsy.* C. Norris. Am. Jour. Clin. Path. 4: 24, 1934. The pathologist who would do medicolegal necropsies must be experienced in necropsy work, but he will have to depend on other specialists, such as the toxicologist, for part of the examination. The public and the legislators who represent them, must be educated to the importance and the extent of medicolegal legislation.—*M. Warwick.*

*Performing the medicolegal autopsy.* A. V. St. George. Am. Jour. Clin. Path. 4: 32, 1934. The operator in these cases must be a detective as well as a pathologist. External inspection is most important. Many causes of death such as angina pectoris, diabetic coma, insulin, shock, and acute alcoholism show nothing at necropsy and this should be kept in mind. Cases of deaths induced by homicide, suicide, or accident may be grouped under the following heads. 1. Crime of violence. 2. Sex crimes. 3. Poisoning. 4. Traumatic accidents. 5. Stillbirths. 6. Accidental deaths.—*M. Warwick.*

*Pathological anatomy of death by drowning.* E. L. Miloslavich. Am. Jour. Clin. Path. 4: 42, 1934. The body surface is pale and the blood usually not clotted. There may be hemorrhages in the soft tissues of the neck. The lungs are large with well rounded edges. They pit on pressure and the cut surfaces do not contract. There is less fluid in them than in true edema. The pleurae show emphysematous projections with ballooning. The lungs do not collapse. The bronchi show a congestion of the mucous surfaces and much mucous secretion in the lumens. There may be many small air bubbles and froth may exude from the mouth and nose. Many foreign particles such as weeds or gravel may be there, but they may have entered after death and do not have significance unless they are in the bronchioles and alveoli. All characteristic changes disappear after putrefaction.—*M. Warwick.*

*Toxicology in medicolegal autopsy.* A. O. Gettler. Am. Jour. Clin. Path. 4: 50, 1934. Must have a fresh, non-embalmed body for satisfactory toxicological examination, and the examination must be performed by someone who has had special training and experience. The pathologist must know what material to reserve for the examination and how to care for it, for example, in clean glass containers. It is well to remember that more than 0.25 per cent of alcohol in the brain or spinal fluid means intoxication.—*M. Warwick.*

*Medical examiner's findings in deaths from shooting, stabbing, and asphyxia.* H. S. Martland. Am. Jour. Clin. Path. 4: 66, 1934. The body must be viewed where it lies. Careful directions for the undressing and trained observation of the body are given and also detailed findings to be found in the various types of death.—*M. Warwick.*

*A new table.* H. C. Schmeisser and I. L. Scianni. Jour. Tech. Meth. 13:

64, 1934 (March). A table of veined marble is described. This has a drain at one end. It is specially good as a background for photographs.—*M. Warwick.*

*Autopsies under indecent conditions.* Notes. Comments and abstracts. *Lancet* 1: 385, 1934. This is a protest against doing necropsies in unsatisfactory surroundings such as very small undertaker's establishment, private homes and such. A plea for a central mortuary is made.—*M. Warwick.*

*The Westminster Mortuary.* Notes, comments, and abstracts. *Lancet* 1: 384, 1934. A description of the model mortuary in Westminster. It has a kelinator ice box with room for 9 bodies and the extracting fans are put in motion by the closing of the door. Four necropsies can be done at the same time. Heat and ventilation are electrically controlled.—*M. Warwick.*

*Postmortems—Their object, time, and place.* E. F. Hoare. *Jour. State Med.* 42: 347, 1934. Postmortem examinations are a matter of public health and do much besides ascertaining the cause of death. Death certificates without necropsy are only doctor's opinion. We suffer from many diseases which give no outward or visible sign. If patients die of some sudden unknown cause, coroner may demand a necropsy, to determine not the medical cause of death but how he died. The public must be won over to the idea of necropsies if many more are to be done. Physicians also should change their attitude to them. The best time to do a necropsy is when it is the public's interest, secondly when it is the interest of the deceased's relatives and third, when it causes the least distress and concern to others. Country not now equipped to do necropsies on every death, but more should be done, especially on sudden deaths. It must be performed quickly, efficiently, and under decent circumstances. The first person to do the examination should be a pathologist if he were available, next the local police surgeon and last the patient's own medical man or any local practitioner. The attending physician may be prejudiced by his own diagnosis and if it is a court case, he would be a biased witness. Mortuaries should be used as postmortem rooms, although the two are usually considered to be separate places. Some mortuaries are not suitable but one building should be erected for both.—*M. Warwick.*

*The relationship of the autopsy surgeon and the embalmer.* I. Forman. *Ohio state Med. Jour.* 30: 167, 1934. "By autopsies, with the aid of the microscope, the practice of medicine has been lifted out of the tangled mass of superstition and cultism and has been put on the basis of exact science." Modern medicine rests on dissection of the dead and our knowledge of disease is dependent upon experience gained from necropsies. The skill of the physician depends on his clinical experience checked by postmortem observation of his patients when they die. First necropsy in America in 1639 and early colonial records show about a dozen. The lack of necropsies in this country is the fault of physicians, the public, and embalmers. In the physicians this is due to the lack of appreciation of the important pathologic observations upon which they base their diagnoses; fear of exposure of this ignorance; being too busy



with practice to bother with necropsies or just plain indifference and this last may be due to the fact that the pathologist does not make necropsies interesting. The public objects to necropsies because of emotionalism due to love of family and a fear which is hard to explain, but religion apparently plays very small part. The attitude of the embalmer can be overcome by cooperation on the part of the pathologist and the hospital. The pathologist should leave the arterial tree intact, avoid unnecessary mutilation, confine his incisions to parts of the body covered by clothing and should not allow undue exposure of the body or much levity in the morgue. The hospital can insist on promptness in having the body ready and signing of the certificate. The Ohio society of clinical and laboratory diagnosis has a permanent committee on necropsies with a bureau of complaints which acts as a clearing house for all complaints of the embalmers.—*M. Warwick.*

*Importance of autopsies to surgeons.* Editorial. H. S. Martland. *Am. Jour. Surg.* 26: 1, 1934 (Oct.). Medical examiners sign about  $\frac{1}{3}$  of death certificates in New York City and Essex County. About 50 per cent of these are violent deaths. Many have been treated by surgeons. In many of these necropsy is necessary to establish the real cause of death and form the basis for legal action. Hospitals make a very much worse showing. In 664 hospitals, only 86 or 13 per cent have more than 50 per cent of necropsies. That is why it is necessary to study pathology abroad. Not enough attention is given to the medicolegal necropsy. The relationship of trauma and cancer is yet to be determined. It is foolish to study cancer from operative or biopsy specimens. No new or unusual tumor should be reported without a complete necropsy to rule out the possibility of an unusual secondary manifestation. More medical papers based on necropsy findings should be printed in the medical literature.—*M. Warwick.*

*Autopsies. Questions and answers.* *Hygeia.* 12: 1039, 1934. Answer: Medical profession wants necropsy to accumulate more information on the character of disease and the causes of death. Necropsies are refused because relatives do not understand the character of the examination and they fear mutilation of the body. They have a fear of the unknown, suspicion based on superstition, religious views and exaggerated sentimentality. The American Medical Association has no autopsy committee but the American Association of Hospitals does and collects bibliography on autopsies. Their report is included in the transactions which may be procured for \$2.00. The Council of Medical Education and Hospitals of the American Medical Association encourages the performance of necropsies and issues a bulletin on the subject which may be procured for 10 cents. The National Research Council published in 1929 bulletin no. 73 by George Weinman on "A Survey of the Law Concerning Dead Human Bodies," which may be had for \$2.00. In the *Archives of Pathology*, 9: 1220, 1930, Schultz has an article entitled "The Law of the dead human body." The first necropsy was performed about 1315 but here were very few until 1502.—*M. Warwick.*



*Necropsy percentages.* Abstract of minutes of meeting of Council of Medical Education and Hospitals of American Medical Association. Jour. Am. Med. Ass. **102**: 702, 1934. Cases removed from the jurisdiction of the hospital by coroner or medical examiner, and, in consequence, not available for teaching material for internes, may be deducted from total hospital deaths in computing necropsy percentage. The same is true of bodies legally assigned to qualified educational institutions for dissection.—*M. Warwick.*

*Consent for autopsy.* Medicolegal. Illinois Court of Appeals. Jour. Am. Med. Ass. **103**: 2054, 1934. Widow has a right to consent to necropsy on body of husband in spite of objection of adult daughter.—*M. Warwick.*

*William Osler at the Philadelphia Hospital.* T. G. Schnabel. Medical Life. **41**: 75, 1934. Osler saw a necropsy as "a continued challenge to surmise an assumption, the most directly available means of verifying observation, of correcting deduction." He doubled the number of necropsies at Blockley, used them to teach students, and as a basis for numerous medical papers. His observations were detailed and very thorough. His interest in pathology was "in a manner that kept students stirred up and that, more or less, wedded them to pathology as a fundamental necessity to the practising physician."—*M. Warwick.*

*Approved internship hospitals with highest necropsy percentage 1933.* Report of Council on medical education and hospitals. Jour. Am. Med. Ass. **103**: 580, 1934.—*M. Warwick.*

	<i>Necropsy percentage</i>
1. St. Luke's Hospital, Kansas City, Mo. ....	86.8
2. Bell Memorial Hospital, Kansas City, Kan. ....	83.2
3. Colo. General Hospital, Denver, Colo. ....	81.8
4. Columbus Hospital, Chicago, Ill. ....	81.4
5. St. Joseph Hospital, Kansas City, Mo. ....	80.4
6. Research and Educational Hospital, Chicago, Ill. ....	76.4
7. University of Chicago Clinics, Chicago, Ill. ....	76.4
8. Kansas City General Hospital No. 1, Kansas City, Mo. ....	75.1
9. University of Nebraska Hospital, Omaha, Nebr. ....	73.8
10. Mount Sinai Hospital, Philadelphia, Penn. ....	72.2
11. St. Elizabeths Hospital, Washington, D. C. ....	72.7
12. State of Wisconsin General Hospital, Madison, Wisc. ....	1.7
13. University Hospitals, Minneapolis, Minn. ....	71.4
14. St. Joseph's Hospital, Reading, Penn. ....	70.8
15. Reading Hospital, Reading, Penn. ....	69.5
16. Santa Fe Coast Lines Hospital, Los Angeles, Calif. ....	69.4
17. University of California Hospital, San Francisco, Calif. ....	69.3
18. St. Margaret's Hospital, Kansas City, Kan. ....	68.6
19. Albany Hospital, Albany, N. Y. ....	68.0
20. Grasslands Hospital, Valhalla, N. Y. ....	67.4

—*M. Warwick.*

*Wanted dead brains.* Meyer. "Time," Feb. 1934. The Johns Hopkins psychiatrist urges every one to give their brains to science so that "we may learn what we may dare do in brain surgery, more about the nutriment support of the brain." He also wants to know what we have to be born with and what can be developed by use as well as suffer by misuse and disease.—*M. Warwick.*

*Injuries of physician or his assistants in course of medico-legal necropsies* Renoux. Ann. de Méd. Lég. 14: 407-408, 1934. The legal aspect of the injuries of physician or his assistants in the course of a medicolegal necropsy is briefly discussed (according to the French law).—*I. Davidsohn.*

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## NEWS AND NOTICES

The Officers of the Laboratory Section of the American Public Health Association are anxious that all laboratory workers in North America know of the Annual Meeting of the Association to be held in New York City, October 5 to 8.

Every active laboratory worker, whether a member of the Association or not, should plan to attend the sessions of the Laboratory Section. The program will include reports on a multitude of laboratory subjects from sanitary phases of public health to considerations of specific diseases such as cancer. The many scientific institutions of the city will be open for inspection, and authorities in many laboratory fields will be available to answer questions.

Further details of the meeting will be announced to all members of the Association in future issues of the American Journal of Public Health. Non-member laboratory workers are advised to contact the Secretary of the Section for further information.

### BUREAU OF HUMAN HEREDITY

115, Gower Street, London, W.C.1, England

The object of this Bureau is collection on as wide a scale as possible of material dealing with human Genetics. Later, the tasks of analysis of material and distribution of the information available will be added.

The Bureau is directed by a Council representing medical and scientific bodies in Great Britain. It is affiliated with the International Human Heredity Committee, which ensures co-operation in all areas where research is proceeding.

The Council would be grateful to receive all available material from Institutions and individuals, furnishing well-authenticated data on the transmission of human traits whatever these may be. Pedigrees are particularly desired; twin studies and statistical

researches are also relevant. As search workers and others who send in material may in some cases wish to retain the sole right of publication (or copyright) those who so desire are asked to accompany their material with a statement to that effect.

Material should be given with all available details in regard to source, diagnostic symptoms, and the name and address of the person or persons who vouch for accuracy. All such details will be regarded as strictly confidential.

Reprints of published work would be most acceptable. Further, many authors when publishing material may also have collected a number of pedigrees which they have been unable to reproduce in detail. It is the object of the Council that such records, by being included in the Clearing House, should not be lost.

Those wishing for a copy of the Standard International Pedigree Symbols may obtain one from the office.

Announcements in regard to the services undertaken by the Bureau will be published from time to time.

Chairman: R. Ruggles Gates.

Executive Committee: R. A. Fisher, J. B. S. Haldane, E. A. Cockayne, J. A. Fraser Roberts, L. E. Halsey (Hon. Treasurer), C. B. S. Hodson (Hon. Gen. Secretary).

Announcement is made of the formation of The New York State Committee on Economics of Pathology composed of the following: Chairman, M. E. Martin, M.D., 153 Lenox Road, Brooklyn, N. Y.; Stephen E. Curtis, M.D., Troy; James Ewing, M.D., New York; Nathan C. Foot, M.D., New York; Wm. A. Wall, M.D., Cortland; Istvan Gaspar, M.D., Rochester; Ward J. MacNeal M.D., New York; A. L. Peckham, M.D., Poughkeepsie; F. E. Sondern, M.D., New York; Secretary, M. J. Fein, M.D., 50 Green Ave., Brooklyn, N. Y.

This committee will interest itself in a consideration of the economic status of clinical pathologists and their specialty with the hope of crystallizing tangible concerted action. To this end the coöperation of pathologists is sought, that by learning from them the evils which exist and by considering the suggestions

made for their remedy a solution of the problem may ultimately be found.

Another successful Convention has come and gone and those who attended have without doubt carried away from it much which will be valuable in their work.

While the complete report of the convention activities will, as usual, appear in the report of the Secretary in the November issue, a few of the more outstanding high lights are presented herewith:

The Society unanimously and with enthusiasm selected Dr. T. B. Magath as President Elect, and as Vice President, Dr. S. P. Reimann.

The Ward Burdick Medal was awarded to Dr. S. P. Reimann, Director of Cancer Research Institute, Lankenau Hospital, Philadelphia, for outstanding merit in cancer research, this choice meeting with unanimous approval.

The gold medal for the best scientific exhibit was awarded to Dr. H. F. Hunt of Danville, Pa. for an outstanding exhibit on the etiology of eclampsia, and the silver medal was awarded to Dr. B. Steinberg, Toledo, Ohio, for an excellent exhibit illustrating the study of peritonitis.

The reports of both the Public Relations Committee and the Executive Committee were of great interest and evidenced that both of these committees had given prolonged and serious study to various problems confronting the clinical pathologist. While these also will be published in the Annual Report in full it is important to mention this one item:

Both Committees, after due and thoughtful consideration and prolonged discussion, have felt it necessary to emphasize to the members at large the relation of each individual member to the local problems confronting the pathologist in individual communities.

While the Society as a whole may lend moral support to attempts to solve such local problems through its enunciation and reiteration of basic principles, in the last analysis the solution of a local community problem must evolve from local activities. In other words, it is highly essential that pathologists shall

through organization and mutual discussion in their own community group formulate their own attack on any given problem and stand back of it and forward it by their own united and definitive proposals and action.

Local problems are so varied in their aspects and present so many individual ramifications that their solution is impossible by any action as a national society. The Counselors in each state are exhorted to consider the local problems in their state communities and by organization in their own communities to bring about concerted action.

The Annual Dinner of the Society was featured by the presentation of properly inscribed gavels to the Past Presidents of the Society as a memento of their term of office.

Among the matters discussed at the business session was an increase in dues. The business of the Society has grown to an extent necessitating increased expenditure.

In view of this fact, and the fact that, some years ago, the initiation fee was reduced from twenty-five to ten dollars, the Executive Committee recommended an increase in the initiation fee from ten to fifteen dollars and increase in the dues from ten to twelve dollars.

A motion was made from the floor to restore the initiation fee to twenty-five dollars and to make the annual dues fifteen dollars. After discussion an initiation fee of fifteen dollars and annual dues of fifteen dollars were adopted.

In view of the large amount of valuable material sent to the Journal some expansion in size is imperative. To aid in bringing this about an increase in the subscription rate from five to six dollars was unanimously voted.



## BOOK REVIEWS

*Surgical Pathology of The Thyroid Gland.* By ARTHUR F. HERTZLER, M.D., Professor of Surgery, University of Kansas. Cloth, 298 pp., 238 illustrations. J. B. Lippincott Co., Philadelphia.

This latest addition to the series of monographs on surgical pathology by this author without doubt will be received and read with great interest by physician, surgeon, and pathologist alike.

The extensive experience of the author is well reflected in this present volume. If it has led him to have the courage of his convictions, it will likewise render others justifiably chary of taking issue with him on disputed points without careful consideration of the record.

This volume presents, not merely the viewpoint of the surgeon, but the views of one who has studied his patients as a whole.

As Dr. Hertzler says: "The story I write is that of a family doctor who has seen many patients." To which it may be added that he has not only seen them but studied them comprehensively, carefully, and thoroughly. Not only their histories and their symptoms but also the pathology shown in the sections of their thyroids.

The book is not only well written but well and profusely illustrated.

This is a volume which none can read without interest and but few indeed without profit. It can be recommended as a comprehensive and critical survey of a not too thoroughly understood group of diseases concerning the management of which there is far from universal accord.

It will prove useful as a reference text to both surgeon and pathologist alike.

*Eugenical Sterilization.* By the Committee of The American Neurological Association for the Investigation of Neurological

Sterilization. Cloth, 211 pp., \$3.00. The MacMillan Co., New York.

All those interested in this subject—whether for or against—will find this a provocative volume.

As a result of a careful and comprehensive study of this controversial subject the Committee recommends selective sterilization, citing chapter and verse in support of its contention.

The book is well indexed and will without doubt become a standard reference text on this subject.

*Model Curriculum for Training Students in Medical Technology.*

The Board of Registry, American Society of Clinical Pathologists, paper, \$1.25.

Here is presented a model curriculum which well deserves the careful study of all who engage in the training of laboratory technicians.

*Quarterly Bulletin of Health Organisation.* The League of Nations, paper, 171 pp., 65¢. Columbia University Press, New York.

This report is concerned with biological standardisation and from this viewpoint discusses insulin, gas-gangrene antitoxin, diphtheria antitoxin, tetanus antitoxin, and antipoliomyelitis convalescent serum and presents, also, reports on international biological standards maintained at the London Institute for Medical Research and the Copenhagen Serum Institute.

*Medical Aspects of Crime.* By W. NORWOOD EAST, H. M. Commissioner of Prisons, etc. With a foreword by Sir John Simon, H. M. Principal Secretary of State. Cloth, 437 pp., \$6.50. P. Blakiston's Son & Co., Philadelphia.

The author has been concerned with criminals, prison administration, and the medical aspects of crime for thirty-six years and that he has amply utilized the opportunities thus afforded him this book bears witness.

The first three chapters of this book present a panorama of prisons and their administration—or, often, until comparatively

recent times, their maladministration—beginning with the sixteenth century.

This is followed by discussions of the mental aspects of prison administration, attempted suicide, exhibitionism, relation of the skull and brain to crime, cellular imprisonment and detention in association, adolescent crime, suicide from the medicolegal aspect, alcohol and drug addiction, prison labor, sterilization, murder, prison reaction types, and psychological medicine and criminal law.

This book is a contribution of value and importance and well repays reading.

*Clinical Laboratory Diagnosis.* By SAMUEL A. LEVINSON, M.D., Director of Laboratories, Research and Educational Hospitals, Chicago, and ROBERT P. MCFATE, M.S., Assistant Director of Laboratories, Research and Educational Hospitals, Chicago, 877 pp., 157 illustrations, \$9.50. Lea and Febiger, Philadelphia.

Despite the many texts available on this subject this volume will receive a cordial welcome from physician, laboratory worker, and pathologist, presenting as it does an unusually complete presentation of the methods of the laboratory in the study of disease.

Departures from the usual are the inclusion of procedures applicable to legal medicine and toxicology, procedures especially applicable to the problems of pediatrics, skin tests and other biological examinations, and histological technic. A special appendix contains a full outline for conducting a course in clinical pathology. Throughout the book there is a consistent endeavor to correlate laboratory findings with clinical symptomatology, a feature of special interest to the physician to whom—as well as to the clinical pathologist—this book may be cordially recommended.

*The Avitaminoses.* By WALTER H. EDDY, Professor of Physiological Chemistry, Teachers College, Columbia University, and GILBERT DALLDORF, Pathologist to the Grassland and

Northern Westchester Hospitals, New York. Cloth, 338 pp., 29 illustrations, \$4.50. The Williams & Wilkins Co., Baltimore.

As stated in the preface, this book is intended to be a helpful manual rather than a complete treatise. Nevertheless, those who turn to it for information will seldom fail to find that which they seek.

As Ewing says in his foreword: "The book is comprehensive in its discussion of the nature of the various vitamins and their functions as well as other information of a very practical nature."

The volume is divided into two sections. The first is devoted to a discussion of the vitamins and the avitaminoses; the second to methods of assaying vitamin sources and of studying the avitaminoses and also to the vitamin values of foods.

The text is not only comprehensive but an authoritative discussion.

It may be heartily recommended to physicians, physiologists, and pathologists as a standard reference text.

*Bright's Disease and Arterial Hypertension.* By WILLARD J. STONE, M.D., Clinical Professor of Medicine, School of Medicine, University of Southern California. Cloth, 352 pp., 31 illustrations, \$5.50. W. B. Saunders Co., Philadelphia.

The importance of the diseases included under the term "Bright's disease" is apparent from the fact that among the causes of death in the United States they occupy third place.

In this volume, the author presents the results of clinical studies over more than twenty years, the result being a book which may be read with interest and profit by physician and pathologist alike.

In the twenty chapters of this book will be found a comprehensive survey of all the phases of renal disease bearing ample evidence of not only extensive but critically evaluated clinical experience. The final chapter consists of the autopsy abstracts of 140 cases of varied types.

Each chapter is concluded by a summary of its salient features and conclusions. There is an author and also a general index.

This book may be recommended as a valuable and informative text.

*Disinfection and Sterilisation.* By ERNEST G. McCULLOCH, Biological Research, Pennsylvania Salt Manufacturing Co., formerly Professor of Bacteriology, Alabama Polytechnic Institute. Cloth, 525 pp., 53 illustrations, \$5.50. Lea and Febiger, Philadelphia.

This book should prove invaluable to all who are interested in or concerned with problems related to sterilisation or disinfection for this volume is as complete and comprehensive survey of the subject as it has been the fortune of this reviewer to see.

Here will be found a scholarly and well conceived account of all the varied methods of disinfection and sterilisation with a critical evaluation efficiency and applicability. Not only are the methods and agents of the laboratory discussed but also those used in the treatment of disease.

As a reference text for sanitary officers, laboratory workers, and physicians alike this text deserves and will doubtless receive a cordial reception.

*Autopsy Diagnosis and Technique.* By OTTO SOPHIR, M.D., Associate Professor of Pathology, University of Illinois, Medical School, with a foreword by Ludvig Hektoen, M.D. Cloth, 342 pp., 65 illustrations, \$5.00. Paul B. Hoeber, Inc., New York.

As Dr. Sophir says in his preface: "What a handbook of 'bed-side diagnosis' is for the clinician, this manual is intended to be for the performer of an autopsy."

Even the most cursory perusal of this text suffices to show that it is based upon an extensive and well digested experience and that it well deserves a place in the library of the pathologist, the coroner's physician, and especially the practitioner for whom an autopsy is an exceptional occurrence.

While the author did not intend to write a text on medico-legal autopsies, emphasis on medicolegal aspects is stressed throughout and particularly in cases of sudden (unexpected) death.

This book may be recommended as a competent and well written text based upon extensive experience.

As Dr. Hektoen says in his foreword: "It tells competently how to make autopsies and how to study their revelations."

*Juvenile Paresis.* By WILLIAM C. MENNINGER, M.D. Cloth, 199 pp., 16 figures, \$3.00. The Williams & Wilkins Co., Baltimore, Md.

This volume is based upon a study of 43 cases personally studied by the author and 610 cases recorded in the literature and is, probably, the most comprehensive study of juvenile parasis yet presented under one cover.

An extensive bibliography of the subject is appended. This book will take its place as a standard reference text and may be recommended as an authoritative study of a rather rare condition.

*Diseases of the Coronary Arteries and Cardiac Pain.* Edited by ROBERT L. LEVY, M.D., Professor of Clinical Medicine, College of Physicians and Surgeons, Columbia. Cloth, 445 pp., 90 figures, \$6.00. The Macmillan Co., New York.

In this volume, under the editorship of Dr. Levy, fourteen contributors present the available knowledge of affections of the coronary arteries and cardiac pain.

The scope of the volume is indicated by its main subdivisions:

Part I: The Coronary Circulation: The Anatomy of The Coronary Vessels, Joseph T. Wearn; The Physiology of The Coronary Circulation, Carl J. Wiggers; The Pharmacology of The Coronary Circulation, Fred M. Smith; The Pathology of the Coronary Arteries, William C. Von Gluhn.

Part II: Cardiac Pain: Mechanism, James C. White; Physiology, Carl J. Wiggers.

Part III: Clinical Features of Diseases of The Coronary Arteries and Cardiac Pain; Statistics, Louis I. Dudlin; Arteriosclerosis, Including Thrombosis of The Coronary Arteries, Robert L. Levy; Syphilis of The Coronary Arteries, Robert L. Levy; Less Common Affections, William J. Kerr; Clinical Significance



of Cardiac Pain, Paul D. White; The Electrocardiogram In Coronary Disease, Frank N. Wilson.

Part IV: The Medical Treatment of Disease of The Coronary Arteries and Cardiac Pain, Robert L. Levy.

Part V: Surgical Treatment, discussed by Jones C. White, Herman L. Baumgart, and Claude C. Beck.

This volume may be recommended to the pathologist, physiologist, and practitioner as an authoritative, outstanding, and comprehensive presentation destined to take its place as a valuable reference text.

## EDITORIAL

### HOW ABOUT THE TUBERCLE BACILLUS IN THE BLOOD?

Among the various and sundry questions asked of the clinical pathologist, one frequently repeated when a diagnosis of tuberculosis is under consideration is, "How about the presence of tubercle bacilli in the blood?" Although such a question always deserves recognition, it must be intelligently answered in the light of the extensive recent investigations which have served to clarify the problem of tuberculosis as a whole and that of tuberculous bacteremias in particular.

Tuberculous bacteremias have perplexed some of the ablest students of tuberculosis and medicine, primarily because these students did not realize the limitations of their methods and because they did not appreciate the natural pitfalls in solving any problem involving the determination of the presence of tubercle bacilli. The simply determined morphological characteristics of this organism gave precedence for the time being to the more important but complex biological qualities of pathogenicity.

Even though facts were available before 1930 to controvert the catholicity of the pathogenic tubercle bacillus, this fascinating theory was still attractive and only required the spark of the newly developed sensitive culture methods to re-embroil us in the unbelievable conception that tuberculous bacteremias were responsible even for non-tuberculous disease manifestations of vague nature. Now after six years of intensive and elaborate studies on man and animals, with culture and animal tests, we are ready to venture a practical opinion.

As early as 1933<sup>1,2</sup> we ventured an opinion, based on the examination of the blood from normal and tuberculous individuals, that human or bovine tuberclobacteremias do not exist in the ordinary case of pulmonary tuberculosis and that the presence

of tubercle bacilli in the blood is far from being a common event in spite of the fact that occasional embolic showers from disintegrating tuberculous foci may be liberated, in which case this embolic material is rapidly removed from the circulation. In the terminal periods of the disease the embolic showers become more numerous and bacilli may then be found more frequently in the blood, but they do not multiply there. These facts were also born out by appropriately performed and evaluated animal experiments.<sup>3</sup>

At the same time the British Medical Research Council recorded their conclusions in a special report.<sup>4</sup> They found tuberculous bacillemia to be far less frequent than claimed and the disease had to assume a phase accompanied by extensive lesions or by actual generalization in order to be of an intensity sufficient to be detectable by present methods. It may be present in five to ten per cent of severe, advanced and progressive cases of pulmonary and in thirty to forty per cent of cases of miliary and meningeal tuberculosis, while postmortem heart blood of patients dying from tuberculosis may reveal tubercle bacilli in about fifty per cent of the cases. Non-tuberculous cases never show evidences of tuberculous bacillemia nor are there any sound reasons for supposing that the tubercle bacillus plays any essential rôle in their etiology. Further, they conclude that "there is, moreover, no reason to believe that the isolation of the tubercle bacillus from the blood is likely to be of any practical value in the early diagnosis of tuberculosis." A valuable feature of this report to the clinical pathologist is the pointing out of the common fallacies attending the demonstration of the tubercle bacillus.

Another pertinent recent review of tuberculous bacillemia is found in the German literature<sup>5</sup> which summarizes the findings as follows: of 10,632 tuberculous cases examined, 101 were positive while of 1641 non-tuberculous cases, 8 were positive; and postmortem blood from tuberculous cases was found positive in 154 of 234 cases. The conclusions drawn are that a tuberculous bacillemia is present in only one per cent of tuberculous individuals as determined by various culture methods and in

four per cent by animal test. "Tuberculous bacillema without tuberculosis does not exist." The discrepancy between the animal and culture tests reported in the foregoing statistics obviously cannot be used for considering the animal test superior to the culture test since the foregoing data was obtained under conditions unfavorable to exacting bacteriologic requirements usually fulfilled by the clinical pathologist and his trained staff.

It is obvious from all the information now available that the routine performance of blood tests for tubercle bacilli would be redundant and that the counsel of the clinical pathologist is here again required to determine when the test deserves consideration, if at all, as an etiologic diagnostic criterion in the individual case. Then, also, it should be performed by an accredited technic suited to the purpose of disclosing small numbers of tubercle bacilli and in a manner beyond criticism whether using the culture test,<sup>6</sup> the animal test,<sup>4</sup> or both.

—H. J. CORPER.

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## THE FUTURE OF PATHOLOGY\*

ROY R. KRACKE†

Fully conscious of the hazards of prophesy it is with some hesitation that I attempt to discuss the future of pathology, but in this time of great changes in social and economic forces, it seems appropriate to attempt an evaluation of our status. I would like to consider particularly the scope of work encompassed in our specialty, our relation to other medical specialties, our place in the general scheme of medicine, the factors that have contributed to the present status of pathology and, from these, attempt to arrive at some conclusions as to what the future may hold for us.

I shall attempt to discuss this question truthfully and impartially, although in so doing I am well aware of the fact that most of you will not agree with everything I may say and some of you may not agree with anything, since this discussion involves a considerable number of controversial issues. I would like to emphasize further that my remarks are personal and not intended to represent the viewpoint of the officers or the membership of our Society. The opinions that I present herewith have been formulated over a period of years and have been influenced by the actual practice of pathology for ten years, by teaching pathology to students of medicine for an equal period, by service on the American Board of Pathology, by years of activity in this Society, and by years of service on the Registry of Medical Technologists. All of this might sound like a recitation of qualifications as an expert witness. It is not so intended, but merely

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*Note:* Reprints of this address may be obtained from the Secretary's office.

†Professor of Pathology and Bacteriology, Emory University School of Medicine, Emory University, Ga.

emphasizes the various sources which affect the formation of my own opinions.

In this discussion I shall use the term "pathologist" as meaning one who practices all divisions of laboratory medicine, and the term "pathology" as referring to the specialty of laboratory medicine. I can see no reason for the distinction between "tissue pathologist" or "pathologic anatomist," and "clinical pathologist," for the reason that most people who practice one of these also practice the other. In our present plan of medical practice the two have not been and can not be differentiated except in large, highly departmentalized institutions.

Pathology as it is practiced today is one of the youngest of medical specialties since its origin dates only from the time of Virchow. Since that time other activities have grown up with it in the evolution of laboratory medicine; these include bacteriology, hematology, clinical chemistry, parasitology and all other activities now carried out in the so-called "clinical laboratory." As medicine has advanced in its relationship and dependence upon the fundamental sciences of chemistry, biology and physics, so has laboratory medicine assumed an ever increasing importance in the diagnosis and treatment of disease. Therefore, the specialty of pathology today, youthful as it is, includes a wide and varied range of activity, so that it is practically impossible for a single person to become expert in all of these divisions. As in the field of internal medicine, it is impossible for one person to become expert in all branches of clinical practice.

Pathology has come to be an important specialty in the general plan of medical practice. This can be more fully appreciated if one considers the duties of the pathologist in the average first-class hospital. These include not only the scientific activities that have been enumerated above, but also general responsibilities in the determination of the type of medical practice that is being done in the institution, the evaluation of work done by clinical colleagues, general supervision over scientific matters in the institution, service as a consultant to various members of the staff, and stimulation of research activity in which the path-



ologist usually lends aid and counsel to the clinician. The pathologist has become the hub of the wheel of scientific endeavor in the modern hospital. Therefore, no one can take issue with the fact that the pathologist, if he does his duty, has come to be an important individual in the better type of medical institution.

However, there are many types of pathologists. There are those who teach these subjects to students of medicine and who engage, little or not at all, in the actual practice of the specialty and who, in many instances, confine their activities in laboratories of academic seclusion. Then, there is the hospital pathologist who has just been described, who practices all divisions of laboratory medicine in larger hospitals. This type of pathologist includes most of the members of our specialty. There is also the pathologist who practices in a number of smaller institutions, along with which roving duties he may combine the private practice of the specialty in his own office. Then, there is the type of pathologist whose work is limited entirely to private practice, in which most of his activities are directed toward aiding in the diagnostic problems of his clinical colleagues. This type of pathologist is restricted somewhat in his activities since he usually does little or no autopsy work and, therefore, in the course of time he may become only remotely associated with the more strictly fundamental scientific aspects of the specialty. Finally, there is the governmental pathologist whose work may be highly specialized and who usually becomes expert in certain branches of the specialty. Among these are the bacteriologists, who direct State laboratories. It can be seen, therefore, that no one set of rules can apply to all of the variety of workers that are incorporated in this specialty.

Before discussing the probable future of pathology, it is necessary first to evaluate its economic status because this by necessity plays an important rôle in its future. In order to do this, let us take up in order the various influences that have contributed to its present economic status.

First, most pathologists today are employed either on a part-time or full-time salary basis. This has come about mainly in

the evolution of the specialty in connection with the growth of hospitals. Many hospital executives seem to have the impression that the department of pathology in the average hospital today is merely another hospital department, and from the viewpoint of certain hospital administrators, it takes equal rank with the department of dietetics, the pharmacy, the engineering division, the nursing division, the laundry, the housekeeping department, and other divisions of hospital activity. Too often the hospital administrator fails to realize that this is a highly specialized professional activity which in every sense is a part of the practice of medicine. Much of this viewpoint arises from the lamentable desire of hospital executives to operate the laboratories to show an unreasonable profit.

Before preparing this paper I discussed this question at great length with a very capable lay hospital administrator and one who is thoroughly aware of the fundamental problems involved in this question. He states that he does not see how the pathologic activity of a hospital can be conducted in any other way that is satisfactory to all concerned, including the hospital administration, the staff, the patients, and the pathologists. It is not within the scope of this discussion to go into the various plans whereby this may be done except to state that it is done in some institutions with satisfaction to all concerned. For the present it may be stated that this is one major factor which has contributed to the economic instability of this specialty.

The second factor is the activity of various governmental agencies in providing various types of laboratory service to the people. This has come about largely through the creation of a nationwide laboratory service operated by the various States under their departments of health. In some of the States such laboratory work is strictly of a public health nature and is designed for the prevention of infectious diseases. In only a few States is such service restricted to indigent patients. In most of them it is available to all of the citizens. In still others the type of work is not restricted to that of public health nature but includes all phases of laboratory activity. Thus, in certain parts of the State of New York there are State and County subsidized labora-

tories which maintain a complete service to the people of those sections. This service is offered to both the sick and the well, in the home and in the hospital, day and night, and includes not only laboratory examinations of public health interest but other procedures including tissue examinations, preparation of autogenous vaccines, basal metabolism determinations and electrocardiography. This is provided to all citizens regardless of their ability to pay. Some of these counties operate branch laboratories in the various hospitals. Thus, from such examples it can be seen that what was once a deserving project has become abused to the extent that such governmental agencies are actually engaged in the practice of pathology. In such instances, therefore, we see classic examples of the complete socialization of this branch of medicine in these particular areas.

This service apparently has been so satisfactory to the people of those communities and even to the clinicians, that they have been hailed with enthusiasm. Little thought has been given, however, to the ultimate effects of such a program. This type of socialized medicine has received considerable impetus in recent years. I would like to emphasize that *the complete socialization of pathology in some areas of this country may be only the forerunner of the complete socialization of clinical medicine*. Therefore, I would like to point out that our clinical colleagues should be seriously concerned with this trend, which may well develop into an opening wedge leading to provision of clinical services on a similar basis.

A third factor contributing to the economic status of this specialty has been certain activities of practicing physicians in which many of them have employed non-medical laboratory workers to carry out not only their own laboratory work, but also that of their associates, all of which is usually done under the pretext of reduction of overhead office expenses. Some of them, however, have discovered that such an arrangement can be an additional source of income. This results in a most pernicious type of commercial exploitation by a few unscrupulous physicians.

A fourth factor of importance has been competition from non-medical groups. This competition has come about mainly from

laboratory technicians, most of whom are capable of performing satisfactorily many of the common laboratory procedures. However, there has been an ever increasing tendency on the part of some of these workers gradually to enlarge the scope of their activity and to carry out diagnostic work for which they are not qualified. This has resulted in an inferior type of laboratory work and the result of such practices will inevitably be a gradual loss of confidence in laboratory diagnostic facilities. It goes without saying that the physician who patronizes such a laboratory jeopardizes the welfare of his patients. Some county medical societies have corrected the situation in their localities by the passage of resolutions condemning the use of laboratories not supervised by clinical pathologists.

The Board of Registry has done much to correct this situation. The registered medical technologists of the United States today comprise the only group around which professional and ethical safeguards have been erected for the control of their activities. The fact that such a movement is not popular among many laboratory workers in this country is evidenced by the recent wave of legislation that has been promulgated in the States of New York, California, Alabama, and others, tending toward the creation of Boards of Licensure for these workers. In other words, this represents a dissatisfaction on the part of laboratory workers who do not wish to be restricted in their activities and who are not satisfied with the restrictions imposed by the Board of Registry.

One factor that has contributed to the uncertain economic status of our specialty is the failure of many physicians to utilize laboratory diagnostic procedures in their medical practice. They simply get along without them and their patients suffer the consequences. This needs no further comment except to state that this probably partly explains why so many patients leave certain sections of the country and go to other places for medical treatment where such services are provided. It explains why a patient leaves one physician and goes to another better equipped for diagnosis through the aid of laboratory work. Therefore, the physician who fails to utilize laboratory aids in diagnosis

may expect to lose his patient to other physicians who do utilize such services.

The sixth factor contributing to the economic status of our specialty is the attitude that seems so widely prevalent among physicians and hospital administrators whereby it is believed that one with meager training can carry out this work successfully. As a result of this we see a certain number of hospitals who employ young doctors as pathologists, usually on a very meager salary, until this doctor has had a chance to become established in his chosen specialty. We also see young physicians in various fields of medicine who attempt to do laboratory work to tide them over the lean days until they become established in their specialty. It is obvious, of course, that the standard of scientific work in such hospitals is, of necessity, low, and the patient becomes the real victim of such a practice.

A factor of importance in recent years is what appears to be an increasing tendency toward the importation of so-called pathologists from other countries from which they have become either voluntarily or involuntarily exiled. This would not be a matter of great concern if there was assurance that these physicians could qualify in this field, but too often clinicians with little or no training or experience in pathology are coming to the United States and engaging in the practice of pathology at extremely low compensation until such time as they may become established in their respective clinical fields.

Finally, a factor of importance has been that in recent years we have experienced an economic depression. It has been necessary to provide medical service at as low cost as possible. Therefore, many practicing physicians have refrained from the use of what are thought to be expensive aids in diagnosis. However, the omission of these aids has usually proved to be expensive for the patient in the long run.

No doubt, there are other factors that have contributed to the economic instability of this specialty, but unquestionably the ones enumerated above are those that have been mainly responsible. Therefore, if we summarize the situation in pathology today, we find it to be one of the most important branches of



medicine that has undergone a slow and gradual economic decline largely because of the influences named above. It is a specialty regarded by many uninformed physicians as an unnecessary adjunct in the diagnosis and treatment of disease. It has become largely socialized in its relation to governmental activity. In some places it has come to be almost non-professional in its relation to hospital administration. Its economic life has become invaded from all sides, including competition from lay workers.

All of this leads to the consideration of what is the probable future of pathology. In many sections the pathologist is looked upon as the "laboratory man" with the opprobrium that the term seems to imply. Even some people question his professional status. The arrogant clinician may at times look upon him with disdain, and perhaps even with contempt. This attitude may be represented by a quotation from a national popular magazine not so many months ago in which it was stated that the dentists of the country were afraid that they might fall to the level of pathologists and urinoscopists. At a recent sectional meeting of the American Hospital Association one of the topics of discussion at a round table conference was "should the pathologist be a member of the staff?" All of this may convey what appears to be an unpleasant tendency in recent years relative to the esteem in which this specialty has been held. This leads us to a consideration of what is the probable future of pathology and even more important, the ultimate effect on medicine as a whole.

First, I would like to point out that there appears to be a definite decrease in the number of young physicians who are choosing this specialty as their life's work. In the medical centers, including larger hospitals and schools of medicine, we still see young men of great promise who are choosing pathology as their career, but these, in my opinion, are becoming less in number as the years go by. In order to appreciate this, one only has to take a vote among graduating classes of medicine in the various medical schools as to what specialties these young graduates are choosing. It is extremely rare that even one is found who signifies his intention of going into pathology. In questioning



medical students on this point, I have been impressed with the almost invariable reply, "Pathologists never make any money; they usually work on salaries; besides they are only laboratory men and I had much rather be a surgeon or a nose and throat man." Furthermore, if one puts the same query to interns in the hospital he gets the same reply except it is even more accentuated. One has only to witness the reluctance of the average intern when assigned to the laboratory service. He accepts it as an unpleasant chore to be performed until he can get back to the stethoscope and scalpel. Therefore, a question of paramount importance is where new pathologists are to be obtained and even more important, pathologists that are well trained and competent and whose cultural and scientific education stands on a par with that of other specialties of medicine. Does this mean that pathologists, as we know them today, will ultimately become extinct? Or does it mean that in the years to come this specialty will become crowded with men who are incapable of success elsewhere? If so, the answer is plain. It means that the pathologist of the future will occupy a position of even less importance and that his economic status will be even more precarious than it is today. It would mean also that the general profession of medicine will have suffered to an immeasurable extent in its scientific aspects and that the progress of medicine will have been retarded almost beyond conception. Of course, we hope that this is not the picture that will be present twenty-five years from today, not only for the sake of medicine itself but also for the sake of the people who are being treated by our clinical colleagues.

A second possible development of the future is the extinction of this specialty as we see it today. There has been an ever-increasing trend in medical education in recent years to emphasize thorough training in pathology for men who practice the various clinical specialties. This can be seen in the larger institutions in which each clinical division operates its own department of pathology, and pathological examinations are carried out by members of the clinical staff. Any plan that tends to afford the clinician an adequate knowledge in pathology for the

practice of his specialty is one to be encouraged. It has been my experience that men who receive such training are more likely to be fully aware of the value of consultation from the specialist in pathology. Therefore, the future of this specialty may depend to some extent upon the training of young clinicians in pathology because only then do they become fully cognizant of the value of pathologic examinations in competent hands.

It is my opinion that the prospects for the future of pathology are excellent. There is no question but that certain things should be done in order that the specialty may occupy the position of importance that it deserves. First, the pathologist should always so conduct himself in his relation to his clinical colleagues that he should command their respect and his opinion should be one that is eagerly sought. Of course, it logically follows that in order for a pathologist to be a man of this type it is incumbent upon him even more than the clinician to study constantly to keep abreast of developments in medicine. It is usually to the pathologist that the clinician turns to learn of new advances in medicine. Therefore, he must measure up to this responsibility and only then can he command the respect of his colleagues. It is an encouraging trend in our specialty to see the large number of pathologists in this organization who are gathered together to study, to discuss scientific questions, and to acquaint their fellows with the results of their own investigations. It is important to study at the microscope for this means that the pathologist recognizes that he must always remain a student. Pathology is advancing with enormous strides, and he who ceases to be abreast of the times is soon lost.

Secondly, the pathologist should always take an active and energetic part in organized medicine. He should attend medical meetings regularly, be a regular attendant at the meetings of his county medical society and sit willingly at the counsel tables when rules governing medical practice are discussed. He should help to create and formulate trends of thought in medical practice. He should not, as is too often the case, be content to isolate himself in his laboratory and assume the self-righteous air of scientific seclusion. He should not look upon prob-

lems of organized medicine as being those unworthy or beneath his notice. Some of the professors of pathology in our schools of medicine have been too content to enshrine themselves in their academic chairs giving little thought to the problems of their practicing colleagues and to the future problems of the very men they are training. It seems inconceivable that there are some pathologists who are not licensed to practice medicine. Such men have no right to expect much consideration from physicians who are licensed to practice medicine.

Thirdly, the pathologist should become interested in the problems of clinical medicine and he should divorce himself from his laboratory and frequently go to the bedside of the patient. In this way he not only comes to appreciate the difficulties and problems of diagnosis in clinical practice, but he is able more intelligently to apply his knowledge to the problems of diagnosis. Furthermore, by so doing he can convey to his clinical colleagues that he is a physician and that he is capable of rendering worthwhile practical information in the diagnosis and treatment of disease. By so doing he serves his most useful function, that is, he becomes a consultant in the true sense of the word. The pathologist of modern times must go from his microscope and the autopsy table to the bedside. The pathology of today is the pathology of the living patient. It is true that much is to be gained from postmortem studies, but the ultimate gain comes in the translation of such knowledge to the sick patient. This trend is evident in medical schools today, since it is not unusual for men trained in clinical medicine to be appointed to teaching positions in pathology. Therefore, might we not consider that some of the difficulties in this specialty may have developed because of laboratory isolation?

It is important for the pathologist to develop an appreciation of his position in medicine. He should remember that after all the clinician has the patient and, therefore, the clinician holds the economic whip. Around this fundamental fact revolves the entire scheme of medicine with all of its accessories. We should always remember that the patient consults his doctor and that in the treatment of disease the physician calls upon us for aid

and counsel and that in this sense, after all, pathology truly is an adjunct in the practice of medicine. Therefore, there is no reason for the pathologist to develop an attitude of resentment for fancied clinical slights.

It is the duty of pathologists as individuals and as a group to educate clinical medicine to the importance of this specialty. This can be done only by a slow and gradual process. The problems of pathology should be presented before county medical societies, state medical associations, and various clinical organizations as often as possible. There is no one to do this but the pathologist himself. The pathologist should exert his scientific leadership and gain the respect of his clinical colleagues, by conducting clinico-pathologic conferences. Such conferences are capable of elevating the entire level of medical practice. They can be made to be stimulating and of the greatest practical importance by emphasizing "living" rather than "dead" pathology.

Just as in other medical specialties in the past, there has been no system of designating and accrediting properly trained qualified pathologists. The American Medical Association has attempted to do this, but this was partly unsuccessful due to the difficulty of evaluating the training and credentials of many pathologists. Since the American Board of Pathology has begun to operate it appears probable that very shortly we shall be in a position to state definitely who are and who are not pathologists. As a matter of fact, it appears quite probable that the certification of pathologists by this Board may finally be the single most important factor in elevating this specialty to its deserved place of prominence. The standards set by the American Board of Pathology, as regards the minimal requirements for post-graduate training in preparation for a career in clinical pathology, provide our medical educators with a definite and tangible basis for the organization of courses of training for young men who seek such a career. It is important that teachers in our medical schools recognize their responsibility in providing such educational opportunities.

Finally, I would like to point out what may be, through no

fault of our own, the eventual solution to these problems. I have pointed out before that in some areas this specialty is now in a state of semi- or total socialization and in this respect it stands unique among the medical specialties. In recent years there has been much discussion and some activity toward the socialization of medicine. The question that arises now is this: Would the specialty of pathology from an economic viewpoint be any worse off with socialized medicine than it is at present? While the status of some pathologists might materially be improved by socialized medicine, it would be most unfortunate if it became necessary for pathologists to campaign actively for socialized medicine in order that they may stand on an equal economic basis with their clinical colleagues. For the clinician to accept placidly and even encourage the socialization of the practice of pathology is to invite and encourage socialization of all branches of clinical medicine. Therefore, I would urge our clinicians to look upon this specialty as an essential branch of medicine and to bear in mind that the problems of the pathologist are the same as the problems of other medical men.

In conclusion, it can be stated that most of the difficulty with the specialty of Pathology today can be traced to economic ills. These have come about largely through the various influences already discussed. There is no question that much of the trouble is the result of the placid attitude of organized medicine in permitting an important specialty to be "sold down the river of socialized medicine" and there is reason to believe that the specialty stands on its last legs. Few young men are going into it so where will it be tomorrow? Ask the pathologist of today if he plans for his son to follow in his footsteps and the answer tells the story.

All of this is a bad omen for the future of scientific medicine, and there can be no doubt but that medicine will deteriorate when this comes about. The pathologist of today, if he is concerned with the future of scientific medicine, could with justification plead for the institution of federalized medicine as the best means of saving it.



## SPECIFIC ARTIFICIAL IMMUNITY IN TUBERCULOSIS\*

H. J. CORPER, MAURICE L. COHN AND A. P. DAMEROW

Tuberculosis, like leprosy and cancer, has been the enigma of medical investigators. Almost anything can be proved regarding this disease if one uses forced, unpractical, extreme experimentation; or if one restricts his observations to a monocentric viewpoint, such as may be deduced from the study of anatomical disease alone; or from statistical observations alone without evaluating the important factor, the causative agent, the tubercle bacillus. Obviously, the latter can be submitted to quantitative analysis only in the fundamental laboratory.

Previously it has been shown qualitatively that the first contact with virulent tubercle bacilli leads to an immunity, in the sense of a changed or protective reaction to subsequent infection, which benefits the infected animal.<sup>1</sup> With cutaneous infection, we have found<sup>2</sup> that there is a definite chronological development of the tuberculous skin lesion following the first intracutaneous infection with virulent human or bovine tubercle bacilli in the guinea pig, dependent upon the amount of bacilli and their relative virulence for this animal. The chronological sequence of development of the tuberculous skin lesion, resulting from the second and subsequent intracutaneous infections of the same virulent human or bovine tubercle bacilli, and in the same quantity, shows a definite retardation. With small infections, there may occur a complete absence of reaction or lesion at the intracutaneous site of the second and following infections as compared with the normal animal.

The original first virulent inoculation pursues its progressive course, and such virulent immunization, although tried, obviously

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would be dangerous and impractical. In guinea pigs, appropriate previous injections with avirulent human or bovine tubercle bacilli given intracutaneously, subcutaneously, or intravenously, but not enterally, retard the development of subsequent infection with virulent human or bovine tubercle bacilli.<sup>3,4</sup> The effect is evident in the retardation of the disease in the local intracutaneous lesions, the tributary glands, and in the metastatic organic disease developed. The immune effect develops definitely at the point where local macroscopic tubercles are produced by the intracutaneous injection of the avirulent tubercle bacilli. Findings in the dog and rabbit verify those noted for the guinea pig. By analogy and from a comparison of intracutaneous reactions in man,<sup>5</sup> there is no reason to believe that this immunity does not exist in man and that it cannot be artificially produced.

Two decided conceptions existed in the past, opposed to the use of artificial immunization with viable avirulent tubercle bacilli in man, that require consideration. One of these contends that there is a possibility of a ready change from avirulent to virulent tubercle bacilli. This contention, however, with avirulent human and bovine tubercle bacilli is not borne out by recent studies<sup>6</sup> and is based on conceptions contrary to our own experimental findings. It is not possible by any procedure yet outlined to change avirulent human or bovine tubercle bacilli into virulent bacilli readily and consistently.

Primarily, the second opposing conception concerns the question of an increased susceptibility to a second infection with virulent tubercle bacilli; that is, that the first infection with virulent tubercle bacilli is always benign (childhood type of disease), and the second, progressive and malignant (adult or reinfection type of disease). This conclusion is reached primarily from anatomical statistical studies (X-ray) without knowledge of the number of tubercle bacilli eliciting the reaction and without recourse to experimental test. In view of the variable reaction occurring to tubercle bacilli, dependent upon the so-called "allergic and immune state," and the variability of spread and new deposit of tuberculous material in the tuberculous individual

himself, it is obvious that more data is desirable before concluding that there exists a greater susceptibility to a second infection. May it not be a massive, transient ("allergic") intoxication with no significant relation to the final amount of disease caused by the reinfection after the reaction subsides and depending upon massive rather than small spontaneous infections?

In all of our studies with tubercle bacilli and tuberculosis, the outstanding fact noted is that quantitative studies for the elucidation of a problem were almost an absolute requisite for proper interpretation. From such quantitative interpretation, it must be concluded that the specific immunity to tuberculosis is strikingly relative in that it can be overwhelmed by infection with exceedingly large amounts of virulent tubercle bacilli. Likewise, it has been demonstrated that dead or avirulent tubercle bacilli in very large amounts can be highly toxic and produce massive lesions. From quantitative studies, it has also been possible to devise a criterion of immunity, and, what is equally important, a criterion of avirulence. The criterion of a specific immune agent is one that will produce a decided relative protection in the guinea pig (the animal of choice for this purpose) to infection with small or reasonably large amounts of a virulent strain of tubercle bacilli as compared with a control animal, either not protected, or also similarly injected primarily with an equal amount of heat killed bacilli (30 minutes boiling water temperature).

The criterion of avirulence of a strain of human or bovine tubercle bacilli requires that a definite amount, injected into an animal susceptible to the strain of tubercle bacilli in question, should not produce a lesion greater than that produced by an equal amount of non-viable bacilli of the same strain. There must be no evidences of multiplication of the bacilli, injected in appropriate amounts, at the site of inoculation or in the internal organs.

That this is true in the human being, as well as animals, is demonstrated by injecting graded amounts of viable avirulent human or avirulent bovine tubercle bacilli (BGG) and comparing the lesions with those produced by equal amounts of non-viable

tubercle bacilli of the same strain. The results of such intracutaneous injections in the arm of man are illustrated in figure 1 (photographs) in which the lesions were studied over a period of six months. The non-viable bacilli were produced by treating with acetic acid (5 per cent) and finally neutralized and diluted

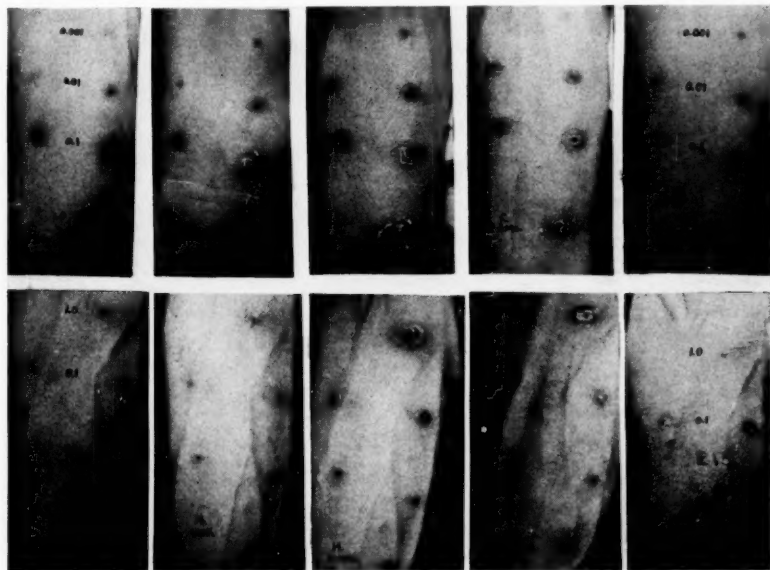


FIG. 1. THE RESULTS OF GRADED INTRACUTANEOUS INJECTIONS OF AVIRULENT HUMAN AND AVIRULENT BOVINE TUBERCLE BACILLI IN HUMAN BEINGS

The upper row illustrates the lesions produced by BCG and the lower row by avirulent human tubercle bacilli. On the right side viable avirulent bacilli were injected and on the left non-viable bacilli. Note the similarity between the lesions produced by the viable and non-viable bacilli after various intervals. Note also the gross similarity between the lesions produced by the avirulent human and bovine bacilli.

before injection. When bacilli were heat-killed or killed by means of other chemical reagents, the results obtained were the same. An observation period covering over two years revealed no progression of the healed lesion, bearing out the fact that in the smaller amounts (0.01 mgm.) where accurate determination

is possible the viable bacilli lose their viability in about four to six months.

Previously, it was shown that following the injection of (non-multiplying) viable avirulent human and bovine tubercle bacilli in amounts capable of eliciting a lesion, the second and subsequent injection of the same amount of viable avirulent bacilli would result in a changed reaction in that a markedly larger

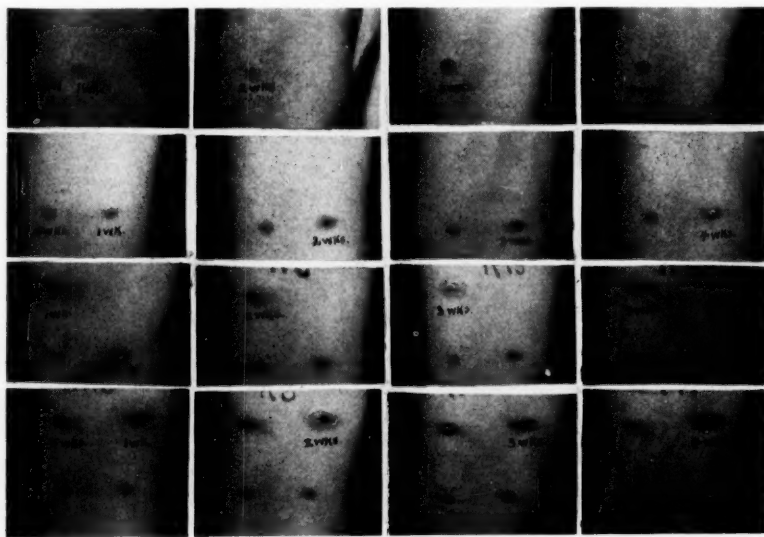


FIG. 2. THE RESULTS OF THE INTRACUTANEOUS INJECTION AT MONTHLY INTERVALS OF 0.1 CC. OF 0.01 MGM. PER CUBIC CENTIMETER OF FINE SUSPENSIONS OF VIABLE AVIRULENT HUMAN TUBERCLE BACILLI IN MAN RELATIVELY TUBERCULIN NEGATIVE

The figures illustrate the findings one, two, three, and four weeks after each injection. Note the greater reaction to the second, third, and fourth injections as compared with the first.

lesion of differing character would result.<sup>5</sup> This consistent change to a greater lesion resulting from the injection of the same amount of viable avirulent human and bovine tubercle bacilli in man is shown in figure 2. This man was relatively tuberculin negative and on careful physical and roentgenologic examination showed no evidences of previous contact with tubercle bacilli.

The change to a greater reaction, which erroneously appears to be a paradox when viewed in the light of the immune reaction, (in which case small infections with virulent tubercle bacilli are completely prevented and dissemination with larger infections are definitely retarded as compared with non-immune controls), is really a manifestation of an increased tissue reaction. It is coincidental with the immune reaction to tubercle-forming amounts of tubercle bacilli, but whether it is synonymous with the immune reaction has not been definitely established. For this reason, it appeared advisable to determine whether the reaction could be elicited only to viable bacilli or whether non-viable bacilli would suffice. Likewise, it was desired to determine whether the changed reaction would occur to both viable and non-viable bacilli, and finally to correlate these findings with artificial immunity. For the purpose at hand, and because comparisons were possible with the elimination of the uncertain multiplication factor, avirulent (human and bovine) tubercle bacilli were used which in previous experiments proved capable of conferring artificial immunity. The experiments were performed in a great variety of manners and with bacilli made non-viable both by chemical means (acetic acid, hexylresorcinol, etc.) and by varying grades of heat, consistent with destroying the viability of the bacilli as controlled by delicate culture methods. The intervals between the first, second, and third intracutaneous injections in guinea pigs were usually four weeks or longer, which in earlier experiments proved to be sufficient for determining a good change in reaction. The intracutaneous injections were given quantitatively (single or double) and in amounts just above the tubercle forming amount (0.1 or 0.01 mgm.). In order to record the findings accurately, photographs in the same magnification were taken at weekly or semiweekly intervals. Two illustrative sets of the photographs are presented in figures 3 and 4. In this work, we found that non-viable avirulent human or bovine tubercle bacilli in like amount do not produce the changed reaction to a second injection of either non-viable or viable avirulent tubercle bacilli regardless whether the non-viable bacilli have been killed by chemical or

physical (heat) means. On the other hand, the first injection of viable avirulent human or bovine tubercle bacilli results in a consistently greater reaction to the second injection of viable and non-viable avirulent tubercle bacilli. Where both viable and non-viable avirulent tubercle bacilli were injected at the same time in the same animal, they produced in equal amounts about equal local reaction.

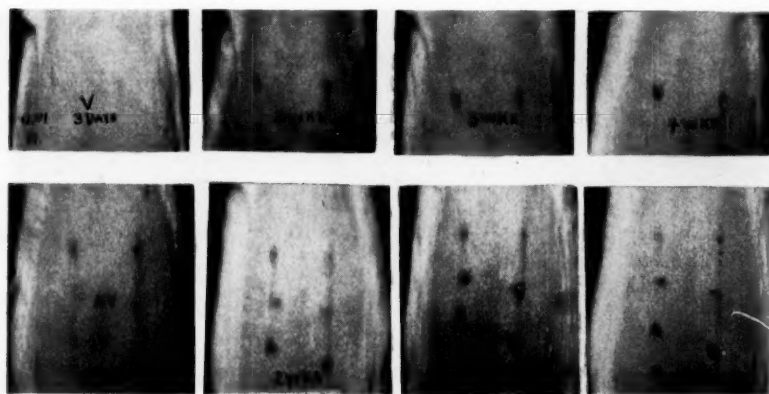


FIG. 3. THE RESULTS OF THE INTRACUTANEOUS INJECTION (IN TWO PLACES) OF 0.01 MGM. OF VIABLE (V) AVIRULENT HUMAN TUBERCLE BACILLI IN THE GUINEA PIG AND FIVE WEEKS LATER A DOUBLE INJECTION OF THE SAME AMOUNT OF NON-VIABLE (HEAT KILLED) AND VIABLE AVIRULENT HUMAN TUBERCLE BACILLI IN THE SAME AMOUNT

Note the decided increase in the second injection of both the viable (V) and non-viable (NV) bacilli in the second row of pictures and the similarity in reaction to the viable (V) and non-viable (NV) bacilli. The pictures were taken three days, one, three, and four weeks after the first and second injections.

In order to correlate further the changed tissue reaction with artificial immunity, a series of guinea pigs were primarily injected with non-viable or viable avirulent human tubercle bacilli either intracutaneously or intravenously (ear vein) and were infected subcutaneously with virulent human tubercle bacilli six weeks later. The non-viable bacilli were produced by means of chemical (acetic acid, methylamine, or ether) or physical agents



(heat at boiling water temperature for 30 minutes, 60°C. for 45 minutes, 53°C. for 12 hours, or 50°C. for 24 hours). In no case did the animals treated primarily with the non-viable bacilli show any retardation in the virulent infection as compared with controls not given a first immunizing injection, while the animals

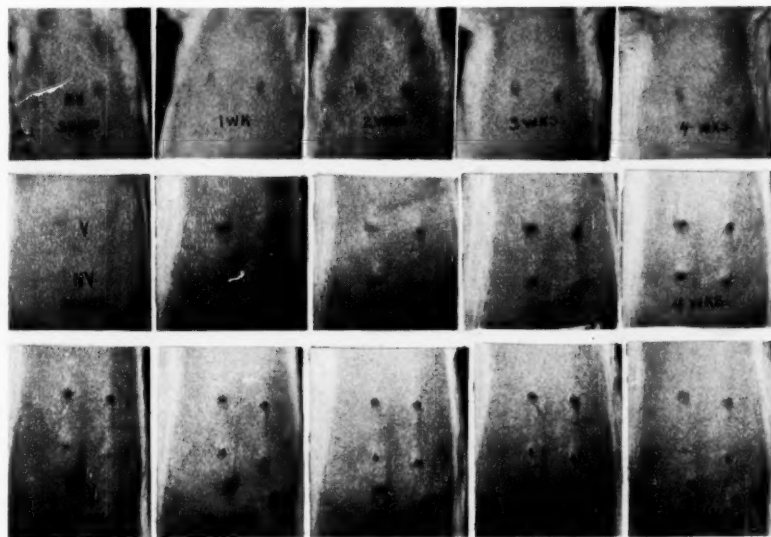


FIG. 4. THE RESULTS OF THE INTRACUTANEOUS INJECTION (DOUBLE) OF 0.1 MGM. OF NON-VIABLE (NV) (HEAT KILLED) AVIRULENT HUMAN TUBERCLE BACILLI (TOP ROW OF PICTURES TAKEN THREE DAYS, ONE, TWO, THREE, AND FOUR WEEKS AFTER INJECTION) UPON THE SECOND INJECTION OF VIABLE (V) AND NON-VIABLE (NV) BACILLI FIVE WEEKS AFTER THE FIRST INJECTION (SECOND ROW OF PICTURES)

Note very little change from the original reaction and no difference between reaction to the viable (V) or non-viable (NV) bacilli. However, when a third injection of viable (V) bacilli is given five weeks after the second injection, this reaction is definitely more pronounced as shown in the third (bottom) row of pictures.

given the avirulent viable bacilli were definitely protected. This is illustrated in an experiment recorded briefly in table 1. Although our findings thus far do not indicate a difference between the changed tissue reaction and the immune reaction, this does not demonstrate that they are synonymous.

In the past, the relation of allergy and tuberculosis has been frequently stressed and correlation between allergy and immunity has been attempted without, however, successfully defining the two. The diagnostic tuberculin reaction is admittedly a protein anaphylactic or allergic response, and yet tuberculin and non-viable tubercle bacilli do not elicit an artificial immunity. Just what part the provocative protein shock response plays in tuberculosis, if any part at all, has often been discussed but has not been adequately elucidated. While studying the effect of

TABLE 1  
COMPARISON OF THE IMMUNIZING ACTION OF NON-VIABLE AND VIABLE AVIRULENT TUBERCLE BACILLI

TREATMENT OF BACILLI USED FOR PRI- MARY INJECTION	BOILING WATER, 30 MINUTES	60°C., 45 MINUTES	53°C., 12 HOURS	50°C., 1 DAY	ETHER, 1 HOUR	ACETIC ACID 5 PER CENT, 3 HOURS	METHYLAMINE 5 PER CENT, 3 MINUTES	VIABLE, NO TREAT- MENT	CONTROL, NO PRI- MARY INJECTION
Tuberculous in- volvement at postmortem 70 days after infec- tion . . . . .	3*	4	3	3	3	4	3	1	3

\* The amount of tuberculous involvement is arbitrarily indicated by the numeral. "1" indicates a local involvement with slightly enlarged glands, and "4" indicates a massive generalized disease. The infecting dose was 0.001 mgm. of virulent human tubercle bacilli, which is relatively large since 0.000,001 mgm. produces a generalized disease. The primary injection of the avirulent bacilli was 1 mgm. in fine suspension given intravenously (6 weeks before virulent infection). The numeral is the average finding from five animals used in each test.

the intravenous injection of virulent avian, bovine, and human tubercle bacilli in guinea pigs and rabbits previously given viable avirulent and virulent bacilli with a view of utilizing the anaphylactic or allergic shock response for differentiating these strains, we were surprised at the lack of effect even following the introduction of relatively large amounts of virulent bacilli. It was impossible by this means to differentiate the strains; and to our surprise, when 1 mgm. of avirulent tubercle bacilli was given intravenously to rabbits and guinea pigs from one to two months

prior to the intravenous injection of 1 mgm. of virulent avian, human, or bovine tubercle bacilli (1 mgm. contains about one billion tubercle bacilli), it was found that these animals suffered no lethal shock from the provocative second injection and lived as long as animals given only the second virulent injection. Those animals, however, cannot be considered to be tuberculous since 1 mgm. of avirulent bacilli do not produce macroscopic tuberculosis when given intravenously.

When this is repeated using 1 mgm. of the same avirulent human or bovine tubercle bacilli intravenously in guinea pigs and rabbits for both the initial and provocative injection, an occasional anaphylactic death occurred only with the avirulent bovine tubercle bacilli (BCG).

In an attempt to sensitize intravenously with larger amounts of avirulent tubercle bacilli and, if possible, to desensitize with repeated small injections, rabbits and guinea pigs were given 5 mgm. of avirulent bovine (BCG) tubercle bacilli (which were chosen because they are slightly more toxic than the human avirulent strains), and in addition they were given, either as a single injection or in repeated small injections of 0.2 mgm. at intervals of one to three days, a total of about 5 to 10 mgm. A provocative injection of 1 mgm. of the same avirulent bacilli in fine suspension was administered a few days after the final small repeat injection of 0.2 mgm. had been given, usually thirty to seventy days later. A typical experiment is illustrated. In this experiment 1 mgm. was given intravenously (ear vein) to seven guinea pigs, and to desensitize they were given intravenously 0.2 mgm. at two to three day intervals until twenty-five injections had been given (about sixty-two days). This was followed by 1 mgm. six days after the last injection and all the guinea pigs survived. After another interval of forty-nine days, another 1 mgm. intravenous injection was given and five of the seven guinea pigs succumbed within two days. The two surviving pigs revealed very little ill effect after the first few days. However, as a control, when eleven guinea pigs were given the 5 mgm. initially and sixty-eight days later were given 1 mgm. intravenously, four succumbed within one to four

days while seven survived with no appreciable ill effect; and forty-nine days later another milligram was given, and five of the remaining seven succumbed. When we consider the susceptibility of these animals to protein anaphylaxis and the size of the injection required to elicit this reaction compared with the size of the animal as well as the mode of administration, it would appear by analogy that man would at least require quite an excessive dose beyond the realm of early disease. With total amounts of 0.1 or 0.01 mgm. of tubercle bacilli, no effects could be elicited even in the guinea pig. In order to carry the study into the realm of remote and rare possibility or those requiring consideration in the advanced human case, we studied extremely large provocative injections of avirulent human and bovine tubercle bacilli in previously injected guinea pigs and rabbits given intravenously 0.01 to 1 mgm. of avirulent bacilli (an amount producing no lesions), or in animals actually tuberculous from the injection of virulent human or bovine tubercle bacilli. The provocative injection for this purpose had to be given subcutaneously because these animals would not readily tolerate intravenous injection of 100 to 500 mgm. even if given as a first injection. In the guinea pigs, this amount of viable bacilli proved lethal within a short time after injection, usually within a few days if the animals had previously been given an intravenous injection of 0.01 to 1 mgm. of avirulent tubercle bacilli or were tuberculous as a result of virulent infection.

In the rabbit, however, when avirulent bacilli in amounts up to 1 mgm. or heat-killed virulent tubercle bacilli (up to 1 mgm.) were given intravenously for the first injection and 500 mgm. of avirulent tubercle bacilli (human or bovine) given subcutaneously as the provocative injection, no appreciable effect was noted. Even in the presence of mild tuberculosis produced by viable virulent human tubercle bacilli or in occasional cases produced by virulent bovine tubercle bacilli, the rabbit survived with little ill effect. However, the usual case of moderate or marked tuberculous disease produced by virulent bovine tubercle bacilli succumbed rapidly to the large provocative injection of avirulent tubercle bacilli.

The results of the foregoing and numerous other experiments are given in summarized form in tables 2 and 3.

It is obvious that the foregoing experiments could not be performed on human beings, and such data as that recorded above must therefore be evaluated with human disease in mind. It may be well to realize that anaphylactic reactions require consideration here and that in spite of the voluminous literature on this subject only recently have Beagell, Bradley, and Ivy<sup>7</sup> consistently induced protein anaphylaxis in dogs by the initial injection of 5 cc. of normal horse serum given subcutaneously and an additional 5 cc. intravenously, followed by a provocative intravenous injection of 10 cc. of horse serum after an interval of sixteen days. Of 61 dogs, 21.5 per cent gave a slight reaction 42.6 per cent a moderate, 16.4 per cent a moderately severe, and 19.7 per cent a severe reaction, while only 1 dog died an acute death. It seems from these figures that anaphylaxis in the dog requires large injections of a foreign protein for elicitation. When we now view the disease tuberculosis in this light, it appears obvious that one of the factors to be dealt with may be the factor of protein hypersensitiveness and its consequences, but it also appears highly probable that this factor only comes into play spontaneously when large amounts of tubercle bacilli are resident in the body, as it is inconceivable that these can enter from the outside to play a part in the course of the disease in man or animals. To conclude from the facts available, because this condition of hypersensitiveness to intoxication may exist from the first contact with viable tubercle bacilli, that the first contact results in greater susceptibility to tuberculosis would appear to take an extremely unwarranted viewpoint. Rather, it would seem more justified to conclude that we are dealing with two alien phenomena or possible remotely related conditions—one, immunity, which can be artificially produced experimentally and which no doubt exists in man as it does in all animal species and is present relatively at least in combating all new contacts with tubercle bacilli whether from without or by dissemination from within; and second, a condition of intoxication induced in the tuberculous individual but requiring the

TABLE 2  
RESULTS OF PROVOCATIVE INJECTIONS OF TUBERCLE BACILLI ON GUINEA PIGS

FIRST INJECTION TUBERCLE BACILLI	PROVOCATIVE* INJECTION NO. 1 AND TIME INTERVAL AFTER LAST INJECTION	PROVOCATIVE INJECTION NO. 2 AND TIME INTERVAL AFTER LAST INJECTION	EFFECT
Avirulent 0.01-1 mgm. I. V.†	Virulent 1 mgm. intravenously 30 to 60 days		No immediate effect. Death few weeks after. Virulent infection as if no primary given
Avirulent 0.01-1 mgm. I. V.	Avirulent 1 mgm. intravenously 30 to 60 days		Occasional death with BCG. No effect with human avirulent
Avirulent 0.01-1 mgm. I. V.	Avirulent 500 mgm. subcuta- neously 30 to 60 days		Lethal within a few days
Avirulent 5-10 mgm. I. V.	Avirulent 1 mgm. intravenously 30 to 60 days		Lethal to about 40 per cent of guinea pigs with BCG
Avirulent 5-10 mgm. I. V.	Avirulent 1 mgm. intravenously 30 to 60 days	Avirulent 1 mgm. intravenously 30 to 60 days	Lethal within a few days. About 75 per cent
Avirulent 5-10 mgm. I. V. (1 mgm., then 25 injections of 0.2 mgm. every 2 or 3 days)	Avirulent 1 mgm. intravenously 6 days		No lethal effect
Avirulent 5-10 mgm. I. V. (1 mgm. then 25 injections of 0.2 mgm. every 2 or 3 days)	Avirulent 1 mgm. intravenously 6 days	Avirulent 1 mgm. intravenously 30 to 60 days	Lethal to majority of animals
Tuberculous guinea pigs	Avirulent 500 mgm. subcuta- neously		Usually lethal

\* These provocative injections were without immediate lethal effect in normal control guinea pigs.

† I. V. indicates intravenously.

absorption of large amounts of tuberculous material such as can occur irregularly mainly by dissemination within and occasionally from without spontaneously, if at all, since the amount of



TABLE 3

RESULTS OF PROVOCATIVE INJECTION OF TUBERCLE BACILLI ON RABBITS

FIRST INJECTION OF TUBERCLE BACILLI	PROVOCATIVE* INJECTION NO. 1 AND TIME INTERVAL AFTER LAST INJECTION	PROVOCATIVE INJECTION NO. 2 AND TIME INTERVAL AFTER LAST INJECTION	EFFECT
Avirulent 0.01 to 1 mgm. I.V.	Virulent 1 mgm. intravenously 30 to 60 days		Occasionally le- thal especially le- thal especially to rabbits injected primarily with BCG
Avirulent 0.01 to 1 mgm. I.V.	Avirulent 1 mgm. intravenously 30 to 60 days		No lethal effect
Avirulent 0.01 to 1 mgm. I.V.	Virulent 10 mgm. subcutaneously 30 to 60 days		No lethal effect
Avirulent 5 to 10 mgm. I.V.	Avirulent 1 mgm. intravenously 30 to 60 days		Occasional lethal effect
Avirulent 5 to 10 mgm. I.V.	Avirulent 1 mgm. intravenously 30 to 60 days	Avirulent 1 mgm. intravenously 30 to 60 days	Occasional lethal effect
Avirulent 5 to 10 mgm. (25 injec- tions of 0.2 mgm. every 2 or 3 days)	Avirulent 1 mgm. intravenously 4 days		Occasional lethal effect
Avirulent 5 to 10 mgm. (25 injec- tions of 0.2 mgm. every 2 or 3 days)	Avirulent 1 mgm. intravenously 3 days	Avirulent 1 mgm. intravenously 30 to 60 days	Occasional lethal effect
Avirulent 0.01 to 1 mgm.	Avirulent 500 mgm. subcuta- neously 30 to 60 days		No lethal effect
Mild tuberculous infection	Avirulent 500 mgm. subcuta- neously 30 to 60 days		No lethal effect
Tuberculous infec- tion moderate or advanced	Avirulent 500 mgm. subcuta- neously 30 to 60 days		Usually lethal

\* These provocative injections were without immediate lethal effect in normal control rabbits.

tubercle bacilli required for provoking a reaction is too great to enter by the ordinary channels of spontaneous infection. It would

be just as radical to conclude from the data available that this intoxication played no part in tuberculosis, for it appears likely that repeated reactions at appropriate intervals may lower the resistance of the advanced tuberculous case as would other detrimental influences.

#### SUMMARY AND CONCLUSIONS

1. The quantitative evaluation of tuberculosis from the standpoint of the bacilli and the disease are essential to an understanding of this chronic disease.

2. As a result of such evaluation and by using avirulent and virulent human and bovine tubercle bacilli, it is possible to define two apparently paradoxical conditions present in tuberculosis which may under certain circumstances exist coincidentally but can be defined as separate entities.

3. One of these conditions, a specific immunity, can be produced artificially by the appropriate use of viable (but not non-viable) avirulent tubercle bacilli and is effective in retarding the development of tuberculosis following small and large virulent infections.

By means of the use of avirulent tubercle bacilli, it was shown that there exists a changed tissue reaction induced by the first injection of viable (but not non-viable) tubercle bacilli which is evident on the subsequent injection of non-viable or viable avirulent tubercle bacilli given in amounts above the threshold of tubercle formation.

4. The second condition, a type of protein intoxication, does not become evident until large amounts of tubercle bacilli are either injected or disseminated internally after a primary contact with viable tubercle bacilli. The provocative reaction is variable dependent upon such factors as are instrumental in anaphylactic shock or reactions.

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## MONILIA INFECTION OF THE LUNGS (BRONCHOMONILIASIS)\*

KANO IKEDA

Monilia infection of the lungs, variously known as bronchomoniliasis, bronchopulmonary moniliasis, etc., represents a chronic and slowly progressive inflammation in which pathogenic monilia is understood to play a cardinal etiologic rôle.

The condition was first recognized by Castellani<sup>2</sup> in 1905 among the tea workers of Ceylon and, for a time, thought to be a tropical or subtropical disease. Numerous cases have since been reported from various parts of the world. The first case in the United States was recorded in 1915 by Boggs and Pincoff<sup>1</sup> in the Bulletin of the Johns Hopkins Hospital. Bronchomoniliasis is no longer considered a rare condition, although the diagnosis must be made with extreme caution since the organism is frequently demonstrated in the secretions of the upper air passages of normal individuals and particularly in the sputum of patients suffering from chronic pulmonary disease, notably, tuberculosis.

Since the infection seldom terminates fatally and only rarely the cases have come to necropsy and since the investigators are, as a rule, interested more in the clinical aspect of the disease and in the organism as its possible etiologic agent, rather than in the pulmonary lesions produced thereby, reports dealing with the pathologic anatomy of this condition have been few and inadequate.<sup>5</sup>

### CLINICAL SYMPTOMS

On the other hand, the clinical history and symptoms of bronchomoniliasis have been fully recorded in a number of excellent case reports, notably by Castellani<sup>2</sup>, Joeke and Simpson,<sup>6</sup> Johns,<sup>7</sup> Stovall,<sup>11</sup> Warr,<sup>13</sup> and others.

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\* From the Pathological Laboratory of the Charles T. Miller Hospital, Inc., St. Paul, and the Department of Pathology, University of Minnesota, Minneapolis, Minnesota. Read before the annual meeting of the American Society of Clinical Pathologists, Kansas City, Mo., May 8th-10th, 1936. Received for publication February 23d, 1937.

Bronchomoniliasis may be recognized clinically in one of three forms, the mild, the intermediate and the severe.

In the mild form, the patient merely complains of a slight cough without temperature elevation or the appreciable physical signs of pulmonary disease. The sputum is scanty and mucoid in character, the cough may continue for weeks or months. The usual diagnosis of chronic bronchitis is made after the routine elimination of early pulmonary tuberculosis. Frequent recurrence of the symptoms is a characteristic clinical feature.

In the intermediate form, the clinical symptoms and physical signs are more exaggerated. There is a persistent low grade fever; the cough is more troublesome; the sputum is muco-purulent and tenacious and may be abundant. There is frequent recurrence of symptoms. The diagnosis of chronic bronchitis, bronchiectasis, or bronchial asthma is usually made although the possibility of pulmonary tuberculosis is never altogether excluded.

In the severe form, two clinical types may be recognized:

In the first type, the patient suffering from the mild or intermediate form of this condition may suddenly develop an acute pneumonia involving a wide area of the lung. This may be a typical form of lobar or bronchopneumonia or as a diffuse inflammation of the lung, in which pyogenic microorganisms usually play an etiologic rôle, while, at the same time, the fungus finds a fertile soil for further multiplication. The patient is acutely ill with elevation of the temperature and all evidence of acute pulmonary infection. This, lasting for a week or more, may subside completely or may be followed by an empyema or other complications of acute pneumonia.

The second type of the severe form may result from a complication of the preceding type, or may represent a progressive low grade infection of a long standing in which no etiologic agents can be demonstrated save the pathogenic monilia and which offers the utmost difficulty in differential diagnosis. A diagnosis of chronic advanced pulmonary tuberculosis is favored in spite of the repeated absence of tubercle bacilli in the sputum. It runs a chronic course with periods of exacerbation. The patient may run a hectic temperature and complain of night sweats; there is a gradual emaciation and loss of weight and strength, and the patient suffers from attacks of dyspnea and severe paroxysmal cough which is worse during the night. The sputum is usually copious in amount, muco-purulent, tenacious, glairy, and often hemorrhagic. It is described as "curdy," "lumpy" or "gruel-like" by various authors. It may suggest an yeast-like or sweetish odor. Occasionally, the secondary, or co-existing bacterial invaders may greatly alter the character of the sputum. Physical examination elicits signs of patchy consolidation and fibrosis and of bronchiectasis or small cavities, usually limited to the lower fields. In the final stages of the disease a gradual failure of the right heart may develop, which is directly responsible for the death of the patient. This last possibility is not mentioned by other authors.

The roentgenogram of the lungs merely may show the usual changes of chronic bronchitis or bronchiectasis. In the severe form, there may be a wide spread shadow indicative of an acute diffuse pneumonia. More frequently, soft, irregular, mottled or feathery shadows with peribronchial thickening and infiltration and intervening areas of emphysema throughout a greater portion of the lungs are described. Cavities may be present occasionally while the pleura may be thickened in chronic severe cases. These constitute a picture often interpreted as chronic pulmonary tuberculosis or chronic mycotic pneumonia.

#### LABORATORY DIAGNOSIS

The mere demonstration of the yeast-like organism of the Genus *Monilia* in the sputum does not constitute the diagnosis of bronchomoniliasis.

The sputum must be obtained directly from the lungs, after having taken every precaution to prevent possible contamination from other sources. It is, as a rule, quite tenacious and mucopurulent, and may be blood-tinged or frankly hemorrhagic during the acute stage. It often simulates a typical "asthmatic" sputum in consistency and general appearance. On close inspection, small, whitish granules may be observed which represent minute masses of the organism. The sputum is persistently negative for tubercle bacilli. There may be a predominance of eosinophiles. On stained smear or wet preparations, the yeast-like bodies, budding forms and sometimes branching filaments of the fungus are demonstrated with comparative ease, especially during the active stage of the disease (fig. 1, A).

The isolated organism should be subjected to the known methods of identification and its pathogenicity determined through animal inoculations. It is generally agreed among the medical mycologists that *M. albicans* is the only species of the Genus *Monilia* which is pathogenic to man and should be regarded as responsible for the development of bronchomoniliasis. It is therefore imperative that the identity of the isolated fungus be established before the significance of the finding is made known.

#### MODE OF INFECTION

*Monilia* is resistant to drying and may live indefinitely in dust and dry environment.

Inhalation of contaminated dust or air probably plays the most important rôle in the transmission of the fungus deep into the respiratory tract. The occurrence of this condition among the tea tasters and the coolies working in the dust of the tea factories in India,<sup>3</sup> among the pigeon dealers who handle dried bird food,<sup>9</sup> among the pedlars of dried fruit and straws in Egypt,<sup>4</sup> all point to inhalation as the most probable means of transmission.

Secondly, monilia may exist as saprophyte in the upper air passages. An yeast-like fungus is frequently observed in the secretions of the nose and



throat and the accessory sinuses. Under favored conditions, the organism may invade the lungs. Likewise, monilial lesions of the skin and mucous membrane elsewhere in the body may be a source of infection.

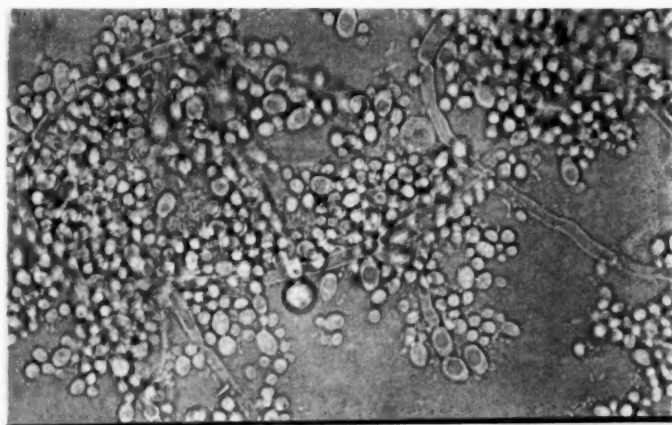
**A****B**

FIG. 1. A. *Monilia albicans* in the sputum of a typical clinical case of bronchomoniliasis.

B. Gross specimen of the lung, after fixation, showing areas of acute pneumonia in which are noted bronchiectasis, emphysema and fibrosis; from a case of clinically undiagnosed chronic non-specific pneumonitis of several years' duration believed to represent an example of bronchomoniliasis.

The transmission from man to man may also be a possibility. More than one member of the same family have been known to be afflicted with this condition.

Since the fungus requires an acid medium for life, there must necessarily be some chemical changes in the otherwise alkaline secretion of the bronchus to favor the continued growth of the organism. This suggests the existence of a pre-existing, primary lesion which must alter the chemical reaction of the tissue before the invasion of monilia can take place.

#### PATHOLOGY

Early pulmonary lesions of bronchomoniliasis have not been described and probably can not be distinguished from those caused by any other form of inflammation. It is possible that a primary bacterial or allergic inflammation of the bronchi or the pulmonary parenchyma may, first, prepare the "soil" for the invasion and growth of monilia which, once planted firmly in the tissue, continues to live a tenacious, semi-saprophytic existence and to mildly stimulate chronic irritation and a resulting low grade inflammation. This conception is supported by the findings in a case of chronic bronchial asthma in whom not only was disclosed the infestation of *Monilia* in the polypoid mucosa from the antrum which had been removed at operation during life but also within the walls of the bronchi and in the regional lymph nodes at necropsy, several months later. This may be considered the pathologic basis of bronchomoniliasis in the early stage.<sup>5</sup>

The continued low grade inflammation of the bronchial wall adds susceptibility to a recurrent acute infection including probably small localized areas of pneumonia which undoubtedly favors the more rapid growth of the fungus. This results in the formation of small abscesses or pseudotubercles in the involved areas such as described by Mendelson<sup>10</sup> who was the only observer to report the small tubercles which "in reality are mycotic tumors which stand out as very prominent masses," in the lungs of individuals who were afflicted with this condition but who died from other causes.

These "mycotic tumors," which are easily reproduced in experimental animals, probably undergo resolution in the majority of cases, leading, as in animals, to complete recovery. Others may break down to form true abscesses which may coalesce to form larger ones. These abscesses may be the basis of the advanced form of bronchomoniliasis or may eventually heal and be replaced by fibrous scar. Only a partial healing is usually the rule and a long continued suppurative and proliferative pneumonitis results in which the major pathologic lesions include the scattered areas of fibrosis, bronchiectasis and abscess formation with intervening and adjacent zones of atelectasis and emphysema (fig. 1, B). In other instances, a chronic pneumonia of unknown origin may become infested with pathogenic monilia which by "adding insult to injury," completes a clinico-pathologic picture of bronchomoniliasis. Overwhelming infestation of monilia in chronic specific lesions such as advanced pulmonary tuberculosis, may actually bring about complete annihilation of the primary etiologic agent, thereby transforming it to true bronchomoniliasis—a speculation which may well be within the realm of possibility.

Microscopically, as in gross appearance, there are no lesions specific for this condition. More conspicuous are the non-specific granulomatous lesions in which equally active are fibroblastic proliferation and cellular exudation, the

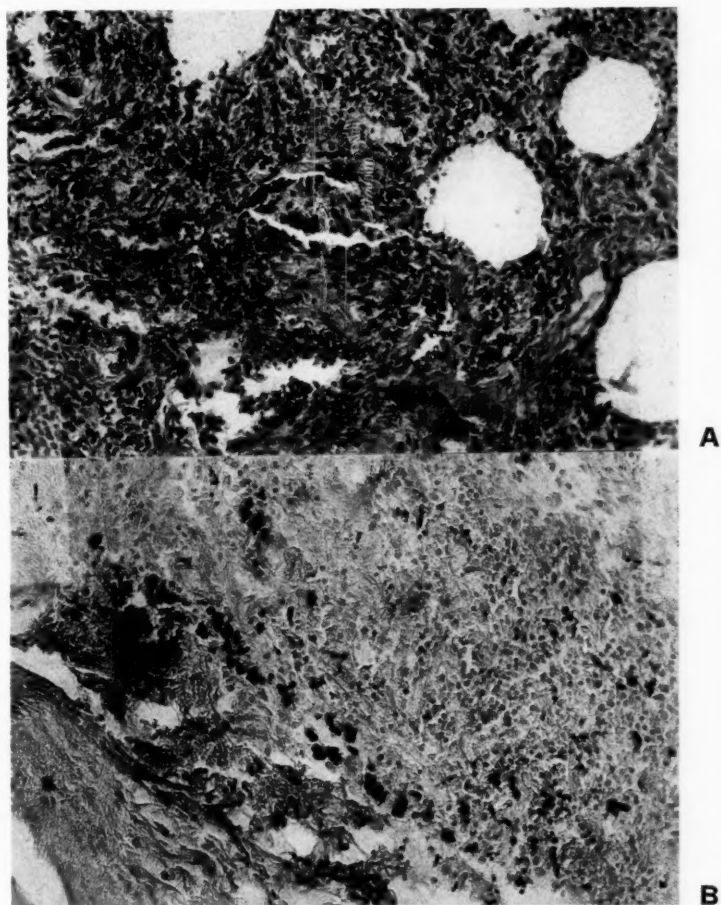


FIG. 2. A. A granulomatous area showing a rich infiltration of plasma cells in which are seen a few distorted air spaces. A section of the lung, shown in figure 1, B.

B. Nests of yeast-like cells along the perivascular space bordering on an area of chronic exudative pneumonia shown in A. (Gram-Weigert stain.)

infiltrates being usually entirely of plasma cells, less frequently of polymorphonuclears or of mixed cellular elements (fig. 2, A). Other changes include the peribronchial and perivascular round cell infiltration, proliferative endarteritis

and the proliferative reaction of the alveolar epithelium, all denoting a long continued, low grade inflammation. Distinct abscesses or bronchiectatic

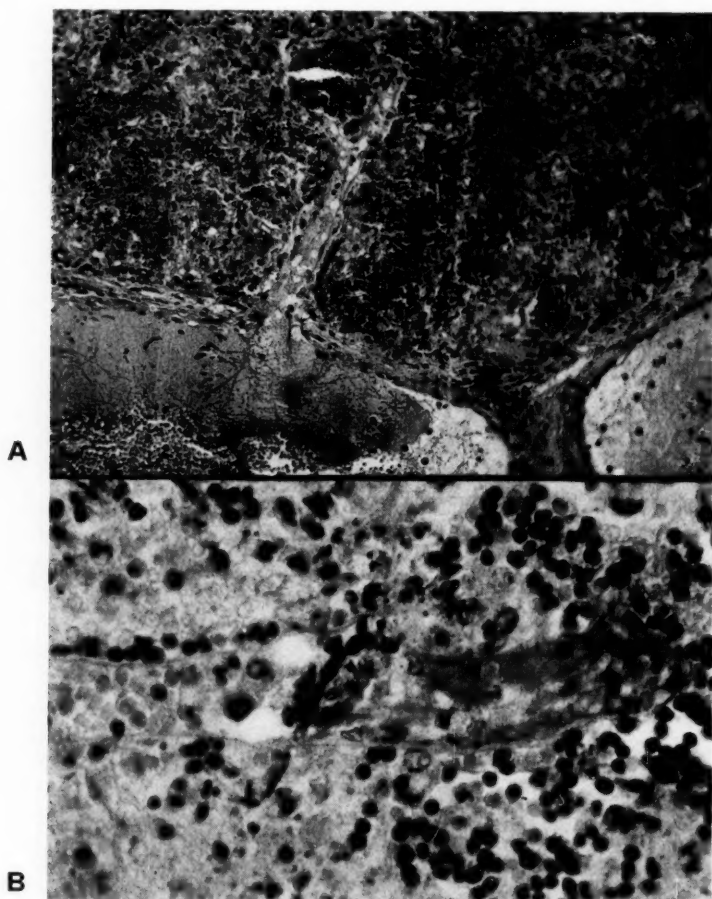


FIG. 3. A. An area of acute hemorrhagic pneumonia produced by intrapulmonary injection of 1 cc. of a saline suspension of *Monilia* culture, showing diffuse extravasation and edema and rapid growth of the organism which penetrates into the lumen of the vessels. Animal killed 2 days after injection.

B. A high power view of a partly thrombosed capillary in a pneumonic area, showing apparent penetration of a mycelium through the wall.

cavities lined with a heavy zone of infiltrates are frequently encountered. There are also areas predominantly productive in type with spreading interstitial fibrosis which obliterates alveoli and causes atelectasis and emphysema of the

adjacent parenchyma. The macrophages are conspicuous in the neighboring, less active areas while foreign body giant cells may be occasionally observed.

The presence of monilia—the nests of yeast-like bodies and mycelia—cannot be demonstrated by the routine H and E stain which accounts for the universal failure to make the pathological diagnosis of this condition. The Gram-Weigert stain brings out the fungus very distinctly and should be employed in every case presenting obscure chronic suppurative lesions of the lungs. The



FIG. 4. A. A section of a lung of a rabbit killed 5 days after intratracheal injection of the organism in suspension. Note several white mycotic "tumors" along the main pulmonary artery.

B. A pleural surface of a lung of a rabbit killed 5 days after intrapulmonary injection of the organism in suspension. Note several white mycotic nodules over the surface.

yeast-like bodies are scattered through the lesions particularly in the walls of the abscess and perivascular spaces (fig. 2, B). They are doubtless taken up by the lymphatics as a pseudo-foreign body. Mycelia are encountered less frequently. The regional lymph nodes are invariably infested with the fungus.

With the finding of histo-pathologic characteristics already enumerated and in the absence of other specific etiologic agents in the lesion and in the light of the positive clinical and laboratory findings, a pathologic diagnosis of bronchomoniliasis may be reasonably made.

## SUMMARY OF ANIMAL EXPERIMENT

The pulmonary lesions were reproduced in animals (rabbits and guinea pigs) experimentally by means of the intravenous, intratracheal and intra-

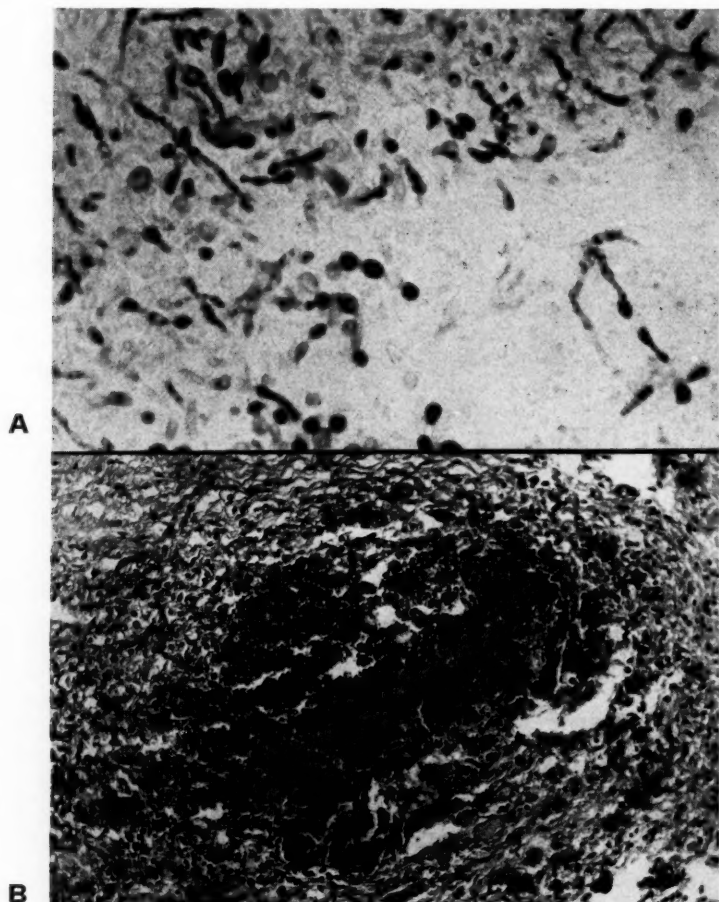


FIG. 5. A. A nest of mycelia in a mycotic "tumor" or abscess obtained by intrapulmonary injection of the organism.

B. A small caseous abscess showing a beginning of calcium deposit in the lung of a rabbit killed 72 days after intrapulmonary injection of the organism.

pulmonary injections of the organism. Saline suspensions of the growth from a young culture of *M. albicans* isolated from the sputum of a typical clinical case of the disease were used according to the technic suggested by Stovall.<sup>12</sup>



The most striking result of the injection, in the majority of instances of rabbits was a rapidly developing miliary cortical abscess of the kidneys with a resulting uremia and death within a week. In these animals, miliary focal abscesses or "tubercles," often microscopic, were also observed in the liver, spleen and meninges. Few animals, on the other hand, developed the characteristic nodular lesions in the lungs. However, in the majority of instances where the intravenous or intratracheal injection was employed the pulmonary lesions were quite insignificant. Small isolated nodules were commonly observed, microscopically, in which the yeast-like bodies were apparently in the process of disintegration. The typical tumor-like nodules were produced in the lungs only in a small number of rabbits treated intravenously and intratracheally. These nodules, as a rule, developed along the main bronchovascular trees as several small, white, discrete, dry masses, well circumscribed and from 2 to 4 mm. in average diameter. They represented, microscopically, a mass of leukocytes and yeast-like bodies mixed with scattered mycelial filaments (fig. 4, A).

The pulmonary lesions were best and most easily reproduced by the intrapulmonary inoculation of the suspension. The direct trauma to the pulmonary parenchyma and the resulting extravasation and serous exudation apparently furnished an ideal medium for rapid growth of the organism thus introduced. This resulted in the development of an acute hemorrhagic pneumonia, with the wide spread dissemination of the monilial filaments which invaded the vessels and caused the formation of thrombi (fig. 3, A and B). With the subsidence of acute inflammation, mycotic tumors developed at the site of pneumonia and along the over-lying pleura (fig. 4, B). These nodules, white, firm and well circumscribed, might coalesce and often break down to form an abscess. Microscopically, the abscess was filled with soft, cheesy material, pus cells and a few scattered collections of monilial bodies and filaments, the latter often in palisade arrangement along the borders of the mass (fig. 5, A). A zone of active fibroblastic and capillary proliferation surrounded the abscess. In later stage, these abscesses underwent calcification and a few became transformed into calcified tubercles (fig. 5, B). A striking observation was the extreme pleomorphism of this fungus in the experimental lesion. This ranged from a small coccid or a long bacillary form to a club-shaped mycelium or a long, sometimes branching, filamentous thread, with various intermediary forms, which, in many instances, can not be morphologically identified as monilia.

#### DISCUSSION

The question of whether bronchomoniliasis constitutes a primary disease entity can not readily be answered. Certainly, the clinical diagnosis of this condition through the mere demonstration of pathogenic monilia in the sputum seems unjustified.

Evidence at hand appears to indicate that monilia is probably a secondary invader in a primary lesion already in existence and that it contributes largely to the continued and progressive activity of that lesion. There are, however, several pertinent observations which may be advanced in favor of the opinion that the fungus may, in a limited sense, at least, act as an etiologic agent: (1) The therapeutic effect of iodides is almost specific. A marked symptomatic improvement, often with complete clinical cure, has been reported. (2) A systemic dissemination of pathogenic monilia appears a possibility as is evidenced by the recovery of this organism from the urine in cases of bronchomoniliasis and by the demonstration of the yeast-like bodies in the regional lymph nodes and in the spleen. (3) No other definite etiologic agents are demonstrated in the pulmonary lesions at necropsy. (4) The condition is undoubtedly consistently overlooked in our routine necropsies, due chiefly to the failure to carry out certain technical details to demonstrate the organism in culture as well as in the lesion. The H and E. stain does not bring out the organism in the tissue. (5) The human lesions can be duplicated in the laboratory animals to which the organism is highly pathogenic. (6) Finally, mention should be made of that unexplored field of allergy, tissue sensitivity or susceptibility which appears to play an important rôle in the pathogenesis of this condition as was suggested by the experiment of Kurotchkin and Lim.<sup>8</sup>

The sequence of events leading up to the development of this condition may be described as follows: The fungus gaining its entrance in the lower air passages begins to multiply in the inflammatory exudate in the presence of bronchitis, bronchiectasis or pneumonia; may penetrate the wall of the bronchus or reaching the alveoli, invade the pneumonic area, to prevent resolution and to continue to multiply, producing a low grade suppurative inflammation; this may continue indefinitely, perhaps for many years, in a vicious circle and predisposes the involved areas to periodic attacks of pneumonia which sooner or later develops into a chronic lesion with areas of fibrosis and suppuration. It would thus appear that a pre-existing, primary lesion in the lung is

essential to initiate the infestation by the fungus. The question of whether specific organisms, such as Tubercle Bacillus, may not be exterminated by the overwhelming presence of monilia and its metabolic products is an interesting speculation.

Certain mycotic infections of the lungs have long been recognized as entities. Blastomycosis and actinomycosis are the common examples. Their known pathogenicity to man and their ability to readily produce local lesions and to cause a systemic invasion and visceral involvement and eventual death have established them as definite clinico-pathologic entities. Monilia, on the other hand, is comparatively less pathogenic to man and often leads a tenacious, saprophytic existence in him. However, given a primary lesion in the lung with attendant bio-chemical changes in the tissue which afford a favorable medium for its unrestrained growth, coupled with the inherent or acquired susceptibility of the host, the fungus may become distinctly pathogenic in the lung of that particular individual and produce a chronic suppurative inflammation known clinically as bronchomoniliasis.

#### CONCLUSIONS

The pathological anatomy of bronchomoniliasis in man and in experimental animals is described and its pathogenesis postulated.

Bronchomoniliasis may be recognized as a pathologic entity.

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# THE OCCURRENCE OF SQUAMOUS-CELL CARCINOMA IN THE LINING EPITHELIUM OF AN OVARIAN DERMOID CYST

WITH A BRIEF REVIEW OF THE LITERATURE\*

M. J. FEIN AND RICHARD HOBART

*From the Pathological and Surgical Departments of the Mountainside Hospital,  
Montclair, New Jersey*

Carcinoma of the ovary is a fairly common malignant lesion, forming from 5 to 8 per cent of the reported malignant neoplasms of the female generative tract. Dermoid cyst of the ovary is likewise seen with considerable frequency, one hundred cases being collected from the records of the Mayo Clinic during a four-and-one-half-year period. But the occurrence of carcinoma arising from the epithelial structures of a dermoid cyst of the ovary is an extremely rare finding, but forty-three authentic cases having been reported up to October, 1933. It has, therefore, been considered justifiable to put on record the case which is the occasion of this paper, and to make a search of the literature to bring the record of authentic cases up to date.

## CASE REPORT

Mrs. S. L., sixty-five years of age, born in Russia, came to one of us with a chief complaint of pain in the lower abdomen, dizziness, frequent urination, exhaustion and constipation. The past history is of no importance.

On physical examination, a large mass is felt on the right side, which is freely movable and occupies a large portion of the lower right quadrant.

On April 27, 1931, the patient was operated upon and a cystic ovary removed. The patient made an uneventful recovery and is still alive six years, post-operative.

## OVARY

*Macroscopical Examination.* The ovary measures 18 x 15 x 12 cm., is rather doughy to the touch and the surface is smooth and pale blue in color. On

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\* Received for publication, June 11th, 1936.

section, the ovary is cystic and the lumen of the cyst is filled with hair and sebaceous material mixed with a chocolate-brown fluid. This cyst occupies most of the ovary and the wall varies in thickness from a parchment-like consistency to about one-and-one-half cm. in thickness. The thicker part of the wall is yellowish-white in color, granular in appearance, and shows small areas of necrosis.

*Microscopical Examination.* The sections taken from the thin parchment-like portion of the wall show squamous epithelium that is atrophied, and in the stroma there are present sebaceous glands, hair follicles, and hyalinized connective tissue. In the lumen, fat and cholesterol crystals are present.

The sections taken from the thickened portion of the wall show a squamous epithelium covering which is unevenly thickened. In the stroma there are collections of masses and columns of cells unequal in size and shape with nuclei that are hyperchromatic and show some mitosis. In the center of many of these masses can be seen hyalinization, keratinization, and the formation of epithelial pearls. Surrounding these neoplastic cells, there is considerable amount of desmoplastic reaction consisting of fibrous connective tissue and round cells.

*Diagnosis.* Dermoid cyst of the ovary (with squamous cell carcinoma in the lining epithelium).

#### PATHOLOGIC ANATOMY

Although dermoid cysts have been found in almost all parts of the body, they occur so much more often in the ovary that the average physician is likely to forget that all dermoids are not ovarian. The structure of the typical neoplasm of this class is so distinctive that once seen it is not easily forgotten. "The contents," says Kouchy<sup>1</sup> "is its most characteristic feature. The yellowish, turbid, oily material containing matted or even felted hair cannot be confused with the contents of any other cyst. When cooled, they acquire a semi-solid, greasy, sticky consistency not unlike vernix caseosa, and not uncommonly containing loose teeth and pieces of bone. Rarely, the fat is found in spheres. This curious phenomenon of globule formation has been attributed by some observers to the churning action of the dermoid by others to the admixture of bloody exudate, since it occurs chiefly in tumors with a twisted pedicle. . . . The typical ovarian dermoid when cleansed of its fat and loose hair, reveals a projection in its cavity. This, known as the plug, pseudomamma, or focus, is covered with hairy skin, and con-



tains the parenchyma of the tumor. . . . The plug or focus is the essential part of a dermoid. Its growth and secretory activity determine the size of the tumor. Its gross anatomy varies from a flat cutaneous area to a structure resembling an acardiac foetus. . . . The rest of the cyst wall is smooth and glistening



FIG. 1. SECTION FROM THIN PORTION OF CYST SHOWING BENIGN PORTION.  
FAT AND CHOLESTERIN CRYSTALS IN LUMEN AND THE LINING  
EPITHELIUM, ATROPHIC SQUAMOUS TYPE

with irregular areas which are reddish in color and wrinkled. . . . Teeth are frequent findings in ovarian dermoids . . . (but) . . . are rarely noted projecting from this part of the wall. . . . Their structure is not unlike that of normal teeth. . . . When bone is present, the teeth are usually embedded in it. . . . Squa-

mous epithelium was found in all cases. The typical dermoid squamous epithelium resembled that found in the mouth. In infected dermoids and in those found in old patients, the epithelium was often shed, or only two or three cells thick. . . . The surface epithelium in depressions on the focus or on the under surface of the bridging type of focus frequently consisted of stratified columnar cells. There was a definite line of transition from the squamous to the stratified columnar type."

#### ORIGIN OF MALIGNANCY IN OVARIAN DERMIDS

Given the peculiar structure of the ovarian dermoid, it is easy to visualize how carcinomatous processes might easily arise there. Yet modern theories of the etiology of malignancy incline strongly to presuppose trauma or irritation as a causal factor in its location and development. According to Lynch and Maxwell<sup>2</sup> carcinoma may develop in an ovarian dermoid (1) by direct extension from a carcinoma of an adjacent organ, or by metastasis from a more distant one; (2) by extension from a carcinoma which has developed in the ovarian tissue not concerned with the dermoid growth, and finally (3) by malignant degeneration of the epithelial structures of the dermoid itself. Nearly all the cases of the third type, which is the only one it is proposed to consider in this paper, are squamous-cell carcinoma.

Counseller and Wellbrock<sup>3</sup> note that the occurrence of primary carcinoma in ovarian dermoids follows the same general rules as those governing that of epitheliomata elsewhere in the body. The fact that most of the reported cases were of women in the period of greatest sexual activity, or just beyond it—thirty to forty-five years of age—would suggest that active sexual function in the ovary might possibly stimulate the growth of the malignant process. There is no direct proof of this, however. Shoemaker,<sup>4</sup> writing in 1890, cites the application of Cohnheim's theory, in determining malignancy in ovarian dermoids. As according to this theory, "all such growths result from the morbid development of embryonal or undeveloped cells which have not been utilized in constructing normal tissues, and . . . have persisted in an otherwise mature organism . . . these em-

bryonic cells may remain inactive for an indefinite time, or may, under proper stimulus, proceed to multiply and develop a tumor." There is a general law that misplaced or anomalous tissues or

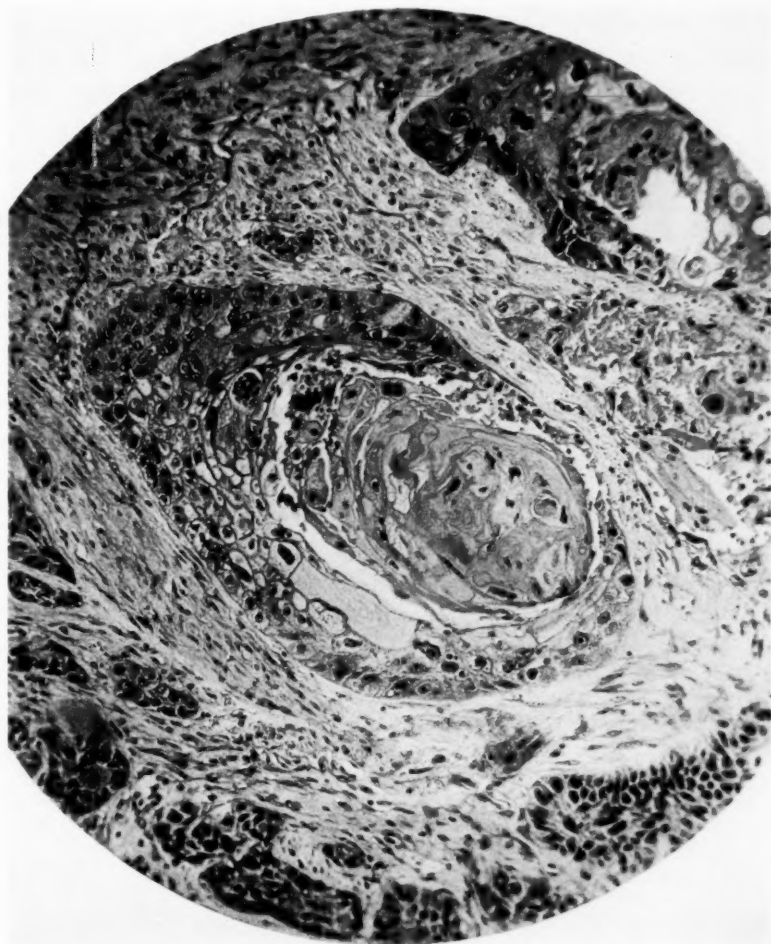


FIG. 2. EPITHELIOMATOUS AREAS, WITH "EPITHELIAL PEARL" IN THE DEEPER PORTION OF WALL

organs are more liable to malignant degeneration than normal ones. Pigmented naevi and angiomas frequently become cancerous, and it has been estimated that the undescended testicle

is two-and-one-half times more likely to develop teretomata than that which has reached its normal position in the scrotum.

This same author also considers the part played by trauma. "Granted that the tissues in a dermoid of the ovary are good soil for the newgrowth, the firm adhesions usually present, the more or less unyielding character of the contents of the cysts make it liable to pressure or injury from the neighborhood of the uterus and the rectum. Conditions, therefore, combine to make the traumatic influence on causation as effective as possible in the case of dermoids of the ovary." All the authors who discuss the chances of malignant development in these ovarian growths, emphasize strongly the wisdom and necessity of making most careful examination of *any* ovarian cyst, no matter how benign and harmless its appearance may be. Diagnosis before operation is out of the question, except in those cases where the growth has advanced so far that any type of treatment is useless. As it is, the mortality is practically one hundred per cent, but a few patients who have survived several years after a through-going extirpation of all tissue which could possibly contain malignant cells, offers a ray of hope to patient and physician alike. Gynecologists should be awake to the likelihood of any dermoid of the ovary being malignant. If examination were always made, no doubt many more malignancies would come to light than have so far been recorded. Spalding,<sup>5</sup> in 1920, made a point of this, and although routine pathological examination is much more common now than it was then, there is still room for much improvement, and every clinician should do his part in seeing that such scrutiny of suspected tissue should invariably be made.

#### HISTORICAL SURVEY

Because carcinoma in an ovarian dermoid has always been so rare a finding, all who have observed cases have hastened to get them into print. Compilations of case reports were made by Clark<sup>6</sup> in 1898; by Williamson and Barris<sup>7</sup> in 1911; by Masson and Oehsenhirt<sup>8</sup> in 1929; and by Counsellor and Wellbrock<sup>3</sup> in 1934. The work of Williamson and Barris was particularly important. They scrutinized the pathological details in the various

reports with the minutest care, and as a result rejected a good many cases which earlier compilers had included. A personal examination of some of these rejected cases seems to the present

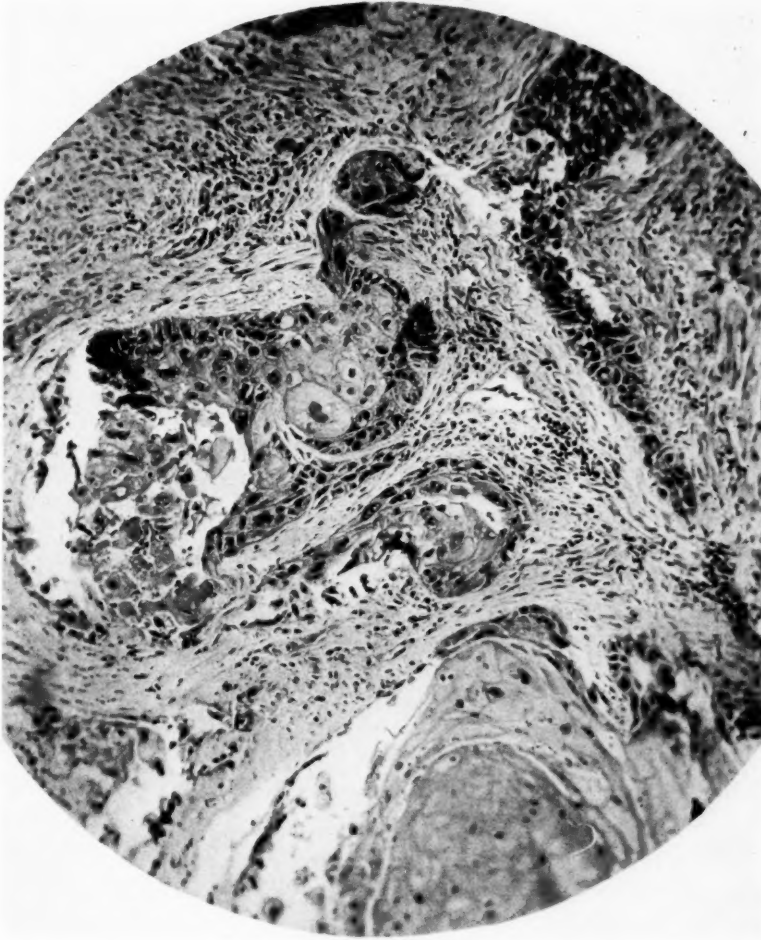


FIG. 3. SHOWING EPITHELIOMATOUS INFILTRATION INTO ANOTHER  
PORTION OF WALL

authors to offer some ground for a difference of opinion. For example, Spalding, in describing his case says, "Immediately beneath the epithelium is a layer of connective tissue and muscle

cells which contains many sebaceous glands and several hair follicles. Several of these glands are invaded with epithelial cell masses resembling basal cell carcinoma. Intermingled with the deeper layers of the connective tissue and extending to the fibrous capsule . . . but not penetrating it, is a carcinomatous mass forming in part solid masses of small round epithelial cells surrounded by a scanty amount of connective tissue, and in part small collections of epithelial cells having an alveolar arrangement." Yet he adds, "It is difficult to decide whether in this specimen we have an adenocarcinoma or a basement cell carcinoma, whether the malignant tumor is primarily in the ovary or comes from a malignant degeneration of the epithelial lining of the teratoma."

Again, Williamson and Barris state that Shoemaker's article was not accessible to them, so they do not include his in their list of reported cases. Masson and Ochsenhirt include the reference in their bibliography, but mention Shoemaker's name in their list of rejections, which reach the rather impressive total of 59 cases. Here is what Shoemaker himself has to say of his case: "A specimen is here presented which exhibits a typical carcinomatous degeneration in the wall of a dermoid cyst removed by Dr. J. Price. The malignant mass is as large as a small fist, and is situated entirely within the limits of the dermoid, which is of large size, its main cavity having contained more than a quart of semi-fluid matter. This has been removed for convenience in preservation, a few hairs being allowed to remain. The carcinoma is quite soft, and easily breaks with a granular fracture. . . . The entire growth, consisting of the cyst and solid cancerous mass, is enclosed in the same capsule, and was readily removed entire from the pelvis." The microscopical work was done by Crozer Griffith and the report is quoted entire. In this we learn, "The portion of the cyst-wall with the newgrowth attached does not differ . . . except at the portion most distant from the interior of the cyst; here the tissue is evidently cancerous. Large numbers of rather small polyhedral epithelial cells with the cell body rather large as compared with the size of



the nucleus, infiltrate the connective tissue to some extent; but still further from the cyst-center they are arranged in dense masses, with few connective tissue septa, forming very large cell-

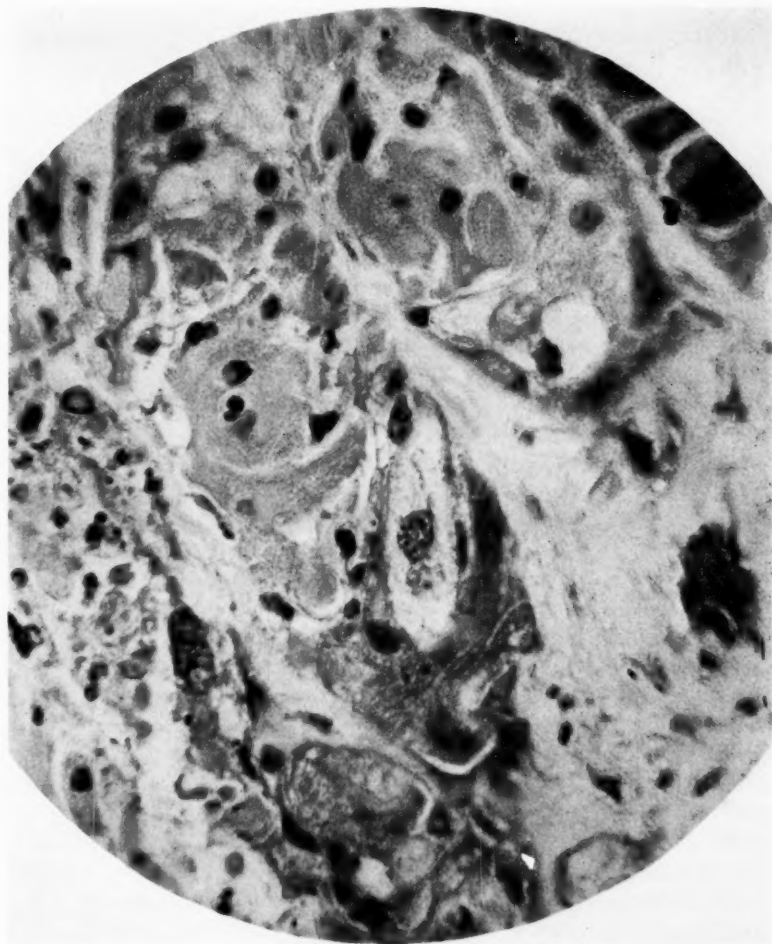


FIG. 4. HIGH POWER, SHOWING HYPERACTIVITY

nesses. The cancerous portion is separated rather sharply from the healthy connective tissue of the wall."

But while thoughtful evaluation of all the pathological evidence might lead to some disagreement with the pronouncements

of these authors, in the main, their conservative attitude is certainly worthy of the highest praise, and the great value of their work—illuminating as it does a rather hazy section in the pathology of the ovary—and the study of dermoids undergoing malignant degeneration—cannot be questioned by gynecologists or pathologists anywhere.

#### COMPILATION OF CASES

The first compilation of cases was made by Clark, who collected six from the literature which he considered authentic, rejected two more which he regarded as doubtful, and added one not before published, being the examination of a specimen seen at Chiari's Pathological Institute, when the author was a student at Prague. Rejecting the cases of Heschl and Von Wahl because of the incompleteness of their pathological descriptions, Clark names Bierman as the first author to report an unquestioned instance of carcinoma arising in the wall of a dermoid cyst of the ovary. The patient who was only twenty-one years old, died from the effects of the tumor before any operative intervention was undertaken. At autopsy the tumor was found to involve both ovaries, though obviously originating on the right. The tumor mass showed two distinct parts—one being composed of cysts filled with characteristic oleogenous fluid, and the other more dense and solid, though in both parts islands of cartilage were in evidence, together with many bony fragments and other debris customarily observed in dermoid formations. The cystic spaces were lined with epithelium, and in those of larger size the epithelium formed typical projections into the underlying tissue, with epithelial pearl formations. In the denser part of the tumor-mass, there were still better defined neoplastic proliferations, practically occupying all of the solid portion.

Bierman's<sup>9</sup> case was published in 1885. The following year a very similar publication was made by Himmelfarb, and in 1887 a third was put on record by Krukenberg. Ten years elapsed before a fourth was published by Tauffer, followed two years later, in 1897, by those of Thumin and of Yamagiva. Clark added his own the next year and published his compilation. When Williamson and Barris made their compilation in 1911, they agreed with Clark in rejecting the cases of Heschl and von Wahl, and likewise decided not to include fourteen others reported since Clark's publication. Of these they omitted Pottion (1887) and Shoemaker, as already mentioned, simply because they did not have access to the original reports. As the present writer has not been able to verify the Pottion reference, this case must still be put on the "not proven" list. The four additional cases which Williamson and Barris added from their own records, as well as those gathered from literature, are all carefully documented, and constitute a valuable addition to the (at that time) scanty literature on the subject of malignant ovarian dermoids.

The paper of Masson and Ochsenhirt, founded as it is on the abundant clinical material reaching the Division of Surgery of the Mayo Clinic, seems destined to remain the classic publication upon our subject. It includes a most complete bibliography, not only of authenticated case reports, but of all literature in any close relation to the occurrence of malignancy in ovarian dermoid cysts, and likewise a tabulation of the thirty-three cases previously published which the authors believed to fulfill all their rather rigid requirements. There is likewise the list of rejected cases, mention of which has already been made. The reader wishing more complete data than is to be found in the present paper is referred to this excellent piece of work, which is not called a "monograph" simply because its most commendable brevity and compactness make it too short to classify as such.

Five years later, two other members of the Division of Surgery of the Mayo Clinic, Counseller and Wellbrock, reported four more cases of malignant ovarian dermoids seen at that institution, with three additional cases from literature, bringing the total of recognized instances up to forty-three. The additional cases were those of Deaver (1931), Ascanio-Suarez (1929) and Delaney (1931). Of the author's own cases the most interesting feature is that two patients were still living—one having survived fifteen years, the operation taking place in July 1917. The other woman was in excellent health one year after operation. Of interest also are the figures which they contribute, compiled from the abundant clinical material supplied by their clinic. "In a total of 408 cystic teratomas surgically removed at the Mayo Clinic, from 1912 to 1931 inclusive, there were seven, 1.7 per cent, which proved grossly and microscopically to be associated with primary epithelioma of the epithelial elements of the cysts. Eighty-eight of the other cysts contained only sebaceous material; 192 contained sebaceous material and hair; seventy-six contained sebaceous material, hair and teeth, and forty-five contained hair, cartilage and bone, and calcareous material."

Since the appearance of Counseller and Wellbrock's paper in 1934, we have been able to find reports of five further cases recorded in literature, brief summaries of which are herewith included. The case of Kleinknecht and his co-workers actually appeared before the Mayo Clinic report, but is not included in their list. The cases reported in *Lyon chir*, xxxii: 453, July-August, 1935, by Villard and Caillot, are the same as those reported by Caillot and Boulez summarized herewith.

KLEINKNECHT, NESSMANN AND GINGLINGER

(Sec. Obst. et Gynec., Strasbourg, Bull. Soc. d'Obst. et de Gynec. de  
Paris, xxi: 365, 1932)

A frail young girl entered the authors' service complaining of a painful abdominal tumor, with peritoneal reaction, which had been present for three days. On palpation, a cystic tumor the size of a six-months' pregnancy was

made out, arising from the left ovary and extending behind the uterus, which was in its normal position. Because palpation was painful in one spot on the superior pole at the right, a pre-operative diagnosis of ovarian cysts complicated by acute appendicitis was made. On opening the abdomen a free dermoid cyst was found in the right adnexa; the left being normal. The appendix gave evidence of old inflammation, but nothing to account for the acute abdominal crises. Further exploration showed neoplastic masses above the aorta against the vertebral column, and likewise upon the anterior border of the liver. These last had greatly distended the liver capsule without breaking it, the great tension thus caused accounting for the extreme abdominal pain.

As both these neoplastic growths were recognized as being metastatic, a search for the primary malignancy was then made by pathologic examination of the removed cyst. The sections showed the characteristic structure of a complex ovarian dysembryoma; multiple cavities were lined with a Malpighian layer, carrying areas of nerve tissue and what appeared to be malformed pulmonary tissue. Among these elements exuberant proliferation was evident, with infiltration of glandular tissue, due doubtless to a malignant transformation of the constituents of the dysembryoma. Similar structures were found in the sections from the liver, offering proof that this growth was a metastasis from the ovarian tumor, a dysembryoma which had undergone cancerous degeneration.

The patient rallied from the operation, but died on the twenty-eighth day. Autopsy showed metastasis widely distributed through the abdomen, though there were no indications of inflammatory reaction and the organs of ileo-cecal region and pelvis were normal.

GAILLOT AND BOULEZ

(Sec. Obst. et de Gynec. de Lyon: Bull. Soc. Obst. et de Gynec. de Paris, xxiii: 564, 1934)

*Case 1.* A woman of forty-eight years, who had never been pregnant, had suffered for two months from intermittent pain in the right iliac fossa. For the past few days this had been augmented by sensations of pelvic compression, and difficulties in evacuating the bladder and lower bowel. Examination showed a large mass in the pelvis, which laparotomy showed to be an enormous ovarian tumor extending into the pouch of Douglas and adherent to sigmoid and rectum. This proved to be a dermoid cyst, filled with hair, which was in an advanced state of degeneration. Respecting the continuity of the lower bowel, as complete an ablation of uterus and adnexa as possible was made. Recurrence from the tissue not removed, took place very shortly, the patient dying within three months. Histologic examination showed no trace of normal ovarian tissue. The sections revealed spinal-cell epidermoid epithelioma in both ovarian dermoid and the extensions into the intestinal region.

*Case 2.* A girl of nineteen years entered the authors' service for anemia, great loss of weight for the previous four months, and an abdominal tumor in evidence for the past two months. Bouts of fever had suggested abdominal tuberculosis; the mass in the abdomen was painful on pressure, and appeared fluctuating. Pulmonary examination was entirely negative. On opening the abdomen a large tumor was exposed, which was adherent to the intestines and extended into the pelvis, filling the pouch of Douglas, and adherent on the left to the pelvic wall. Puncture returned 300 grammes of thick purulent fluid and when the mass was freed, it was revealed as a dermoid cyst of the left ovary, filled with hair, and much degenerated. The posterior wall of the infantile uterus (size of a fig) was involved in the tumor. When a sub-total hysterectomy had cleared away this mass, there were seen in the cul-de-sac of Douglas, upon the posterior wall of the bladder, and on the sides of the cavity left in the pelvis after removal of the cyst, large quantities of neoplastic tissue. As these were very friable, most of them were curetted away, although it was impossible to remove it entirely. Examination of specimens taken from both ovarian cysts and uterine wall, showed identical structure—spinal-cell epithelioma in active mitosis. As the malignant process had broken through the cyst wall and invaded the pelvic cavity where it was evidently widely distributed, the authors gave no hope of ultimate recovery, although their patient was alive at the time the report was made, and had gained somewhat in strength and general health.

PETROWA AND KARAWEA

(Arch. f. Gynäk. cliv: 422, 1935)

A woman of 52 years entered the authors' clinic for treatment of an abdominal tumor. For a year previous there had been pain in the left adnexial region. The pre-operative diagnosis was cyst of the ovary, with malignant degeneration. Laparotomy showed the entire abdomen occupied by a tumor springing from the left ovary. This tumor was of cystic consistency, but from its upper pole sprang another tumor, lobulated and firm in consistency, which was as large as a man's fist. This supernumerary tumor had involved the peritoneum and was attached to the neighboring organs by inflammatory adhesions. During the process of freeing and removing the mass, the wall of the cyst was ruptured permitting a quantity of fatty fluid to flood the abdominal cavity. A satisfactory closure of the operative wound was effected and the post-operative course was uneventful. Microscopically, sections from the wall of the cyst showed a uniform structure of fibrous tissue, but those taken from the pseudomammary portions showed an alveolar structure and squamous-cell epithelium, characteristic of ordinary carcinoma tissue. In various parts of this section cells in different states of maturity could be made out. Epithelial pearls were in evidence. Extensive necrosis had taken place in the center of the focus. The lymph-canals were choked with cancerous proliferation, and there was



much fibrous overgrowth of the stroma. Here and there hyaline areas appeared, bearing a resemblance to old *corpora albicantia*. A section cut from the solid portion of the secondary tumor showed sarcomatous tissue with evidence of rapid proliferation of giant-cells rich in chromatin. A section made a little further on, showed sarcomatous tissue in an advanced stage of disintegration. A section made from the periphery at the point where the solid tumor was given off, showed cancer tissue of exactly the same character as that taken from the focus projection itself. Thus both carcinomatous and sarcomatous tissue were demonstrated in the growth.

#### MERIAL AND DIEULAFE

(Gynec. et Obst., xxxi: 720, May 1935)

An unmarried woman of sixty-three years presented an abdominal tumor, which her physician diagnosed as peritoneal tuberculosis with ascites. Evening rise of temperature and continued loss of weight had been noted for four months. On opening the abdomen, a voluminous cystic tumor was exposed. Punctured before removal, it was seen that the cyst did not arise directly from the ovary as had been supposed previously, but that the fluid had gathered under the serous envelope of a tumor occupying the site of the right ovary. When this tumor had been brought into the wound, it was noted that the right tube was dilated about four centimeters from the uterus by a nodule about the size of a nut. The adnexa of the right side were extirpated, but the uterus and left adnexa, being healthy, were left in place.

On section, the ovarian tumor showed wide zones of typical epitheliomatous tissue; other zones showed cells resembling those of a seminoma. Elsewhere there were regions of leukocyte infiltration, and some sections showed wide myxomatous plaques. In still other regions there were curiously formed cavities in which were found substances resembling chorial villousities. The growth was definitely epitheliomatous and apparently had arisen from a degenerated dermoid cyst. The nodule in the Fallopian tube, proved on section to be a typical dermoid cyst. The authors conclude that the patient had congenitally two dermoid cysts in her genital apparatus; that one of them degenerated giving rise to the massive epithelioma, and that this later growth, giving rise to a great liquid effusion, the fluid, instead of expanding into the peritoneal cavity as is the ordinary course of such cases, remained localized under the limiting membrane of the neoplasm. Thus the free ascites characteristic of ovarian growths, was encysted in the tumor itself. These circumstances made pre-operative diagnosis impossible.

Four months after operation, the patient was in perfect health.

#### SUMMARY

1. Carcinoma of the epithelial lining of a dermoid cyst of the ovary is a rare finding. To date, including the case herewith



reported, forty-nine cases only have been recorded in medical literature.

2. The peculiar structure of dermoid cysts and their location in the ovary seem to offer a congenial site for the development of malignancy. If it were possible to make pre-operative diagnosis early enough, doubtless many more cases would come to light.

3. A list of authoritative papers containing bibliographies and tabular reports of cases is given.

4. An additional case is reported in detail, and brief protocols of five cases published since the appearance of Counsellor and Wellbrock's paper in 1934, are included.

5. The desirability of reporting all such cases is emphasized.

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## HEMOLYSIS OF RED CELLS IN NEPHRITIS IN SAPONIN SYSTEMS\*

FRANK J. C. HERRALD† AND MICHEL PIJOAN

*From The Medical Clinic, The Peter Bent Brigham Hospital*

One of the common characteristics of chronic glomerulonephritis is a continued anemia, for which no adequate explanation has as yet been set forth. To be sure, there is a persistent hematuria, but no one believes this sufficient to account for the degree of anemia present. Brown and Roth<sup>1</sup> and Fishberg<sup>2</sup> offer the plausible theory that the erythropenia is dependent on a suppression of bone marrow function, a hypothesis not, however, substantiated by pathological findings. A chemical etiology for the anemia is suggested by the common inverse relationship between nitrogen and creatinine retention and the hemoglobin content of the blood<sup>1</sup>, but Fishberg<sup>2</sup> has shown this not to be a rule but a mere associated finding.

It seemed to us that one of the first steps in the study of this problem ought to be an investigation of the behaviour in simple hemolytic systems of the erythrocytes from nephritic patients. The resistance of red cells to hemolysis in non-nephritic anemic patients has been studied primarily by the use of hypotonic saline solutions. In such hemolytic systems, however, the cells are necessarily mixed with serum and no attempt is made to compute the velocity of hemolysis or to measure the cells or serum quantitatively.

The present study is concerned with the exact relationship of cell and serum components in standardized saponin systems. The course of this investigation proceeds along two lines, (A) the hemolysis by saponin of washed red cells from nephritic

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† McCosh Bursar, University of Edinburgh.

patients and (B) the effect on hemolysis of serum from nephritic patients.

#### GENERAL CONSIDERATIONS

##### *Principle*

Ponder has shown that during the process of stromatolysis by saponin, hemoglobin and other cell constituents are liberated and that these may interact with the saponin to produce either inhibition or acceleration of hemolysis. Lysin may also be fixed or absorbed at the cell surfaces, just as sensitizing agents or complements are adsorbed. The kinetics of such systems and the quantitative methods involved have been fully presented by Ponder.<sup>4, 5, 6.</sup>

The principle of the method we have employed is that described by Ponder<sup>5</sup>, where the lysin (saponin) is added quantitatively to a known volume of washed red cells in saline, and the velocity of hemolysis noted. This method is convenient and checks remarkable well with the more quantitative time dilution curve system of Ponder.<sup>4</sup>

#### A. HEMOLYSIS BY SAPONIN OF WASHED RED CELLS FROM NEPHRITIC PATIENTS

##### *Apparatus*

1. *Evelyn Photometer.* The technique of determining percentage hemolysis, either by the apparatus described by Ponder<sup>5</sup> or by the Pulfrich photometer<sup>7</sup> has been modified so that accurate red cell counts may be obtained every ten seconds during the course of hemolysis. For this purpose we have used a type of photometer devised by Evelyn.<sup>8</sup>

In this instrument a filter is chosen which will transmit light between any desired wave lengths, depending on the color of the solution in question. In our experiments with red cells Corning glass filters adapted to the apparatus were used. These filters transmit light only between  $\lambda$  630 $\mu\mu$  and  $\lambda$  650 $\mu\mu$ , as at this range light is least absorbed by free hemoglobin in solution. A calibration curve was calculated by comparing actual red cell counts per cubic millimeter (by counting-chamber method) with millimeter deflections of the galvanometer (1 millimeter = 0.027 microampere). Since a suspension of red cells is opaque, while a solution of hemolyzed cells is clear, the former will absorb more light than the latter. The quantity of light passing through a suspension containing cells will, therefore, vary with the number of cells hemolyzed, increasing as hemolysis proceeds.

TABLE 1  
NEPHRITIC PATIENTS. TIME REQUIRED FOR COMPLETE HEMOLYSIS OF RED CELLS IN STANDARD SAPONIN SYSTEMS

NAME	AGE <i>years</i>	DIAGNOSIS	DURATION OF DISEASE <i>years</i>	BLOOD CHEMISTRY	URINARY FINDINGS	EDEMA	BLOOD PRESSURE	HEMA- TOCRIT	R.B.C.	TIME FOR COMPLETE HEMOLYSIS BY SAPONIN <i>seconds</i>
F. T.	42	Chronic glomer- ulo-nephritis	17	Blood urea N 45 mgm. per cent Total proteins 6.7 grams per cent	Albumin LT R.b.c. 15-20 p.h.f. Hyaline and granular casts	-	140/92	26	2,800,000	800
R. M.	18	Chronic glomer- ulo-nephritis	5	Blood urea N 45 mgm. per cent Total proteins 6.0 grams per cent	Albumin ST R.b.c. 8-10 p.h.f. Granular casts	0	190/40	32	4,000,000	500
E. P.	28	Chronic glomer- ulo-nephritis	*	Blood urea N 11 mgm. per cent Total proteins 3.7 grams per cent	Albumin T Hyaline granular casts	---	175/110	24	3,200,000	450
A. C.	17	Subacute glo- merulo-nephritis	1	Blood urea N 16 mgm. per cent	Albumin ST Occasional cast R.b.c. 10-15 p.h.f.	0	120/80	33	3,640,000	620

M. B.	46	Chronic glom- ulo-nephritis	10	Blood urea N 129 mgm. per cent Total proteins 6.4 grams per cent	Albumin T R.b.c. 20-30 p.h.f. PSP O	0	220/110	18	2,500,000	460
D. R.	41	Chronic glom- ulo-nephritis	11	Blood urea N 34 mgm. per cent Total proteins 5.5 grams per cent	Albumin LT R.b.c. 5-6 p.h.f. Granular casts	-	198/128	24	2,800,000	620
C. C.	18	Subacute glom- erulo-nephritis	1	Blood urea N 14 mgm. per cent	Albumin ST R.b.c. 5-6 p.h.f. Hyaline and granular casts	0	140/90	39	3,100,000	540
D. L.	26	Chronic glom- ulo-nephritis	3	Blood urea N 20 mgm. per cent	Albumin ST	-	150/100	21	2,600,000	450

\* Unknown; first seen in 1925.

2. *Hematocrit Tubes.* Standard recalibrated 2 cc. Wintrobe hematocrit tubes were used. Oxalated blood was placed in these tubes and centrifuged for one hour at 4000 r.p.m.

3. *Centrifuge Tubes.* Fifteen and 50 cc. centrifuge tubes which have been carefully calibrated to read in volumes of 0.1 cc. of blood were used.

4. *Evelyn Tubes.* All readings taken with the Evelyn photometer require the use of a 30 cc. standard tube with an internal diameter of 20 mm. The tubes were previously tested as to the evenness of the glass with a standard known solution of hemoglobin.

5. *Pipettes.* All pipettes used for the delivery of blood or red cells in saline suspension were recalibrated for that particular system.

6. *Saponin.* Pure saponin (Merck) was employed. Throughout the course of the investigation we used only saponin from the original stock supply. This precaution eliminates certain discrepancies which may occur with the use of different lots of this lytic agent.

### Method

Ten cubic centimeters of fasting blood were drawn by venepuncture into a dry syringe, quickly transferred to a clean, dry test tube containing 20 mgm. of potassium oxalate, and mixed.

After the relationship of cells to plasma had been determined by hematocrit, 0.4 cc. of whole blood was washed twice by centrifuging with 0.85 per cent saline solution. The supernatant fluid was decanted and the cells made up to a volume of 8 cc. with saline. If the hematocrit reading was the arbitrary normal of 45, then 2 cc. of the 8 cc. suspension were taken, this 2 cc. containing red cells from 0.1 cc. blood. If the hematocrit reading was below 45, more than 2 cc. of the suspension were required and this amount calculated by simple proportion. Similarly, less of the suspension was taken when the hematocrit was greater than 45. This calculated amount of red cells was now taken up in saline solution to a total volume of 19 cc. By making a correction for hematocrit values in this way, the actual cell count varied only between 4,800,000 and 5,000,000 r.b.c. per cubic millimeter. Consequently, the same number of red cells were introduced into the Evelyn tube regardless of the degree of anemia present in the patient. The final solution, therefore, contained, where hematocrit was 45, 2 cc. of red cell suspension, 17 cc. of 0.85 per cent saline, and, to be added forthwith, 1 cc. of saponin, making a total volume of 20 cc. When the hematocrit reading was greater or less than 45, the volume of red cell suspension differed slightly, and the volume of saline had to be adjusted, but in every case the final volume was 20 cc. after addition of 1 cc. of saponin. The amount of saponin in the final system when 1 cc. of 1:500 saponin was added was 0.002 gram.

Two Evelyn tubes were prepared in all cases. To one tube was added 1 cc. of saponin 1:100 which brought about hemolysis in a few seconds. When



complete hemolysis, which is obvious to the naked eye, had taken place, the tube was placed in the Evelyn apparatus and the galvanometer was adjusted for convenience to read 100. The reading of this amplitude was then equivalent to one hundred per cent hemolysis. To the second tube was added 1 cc. of saponin 1:500, and this was placed in the apparatus after gentle shaking.

TABLE 2

NORMAL SUBJECTS AND PATIENTS WITH PRIMARY OR SECONDARY ANEMIA. TIME REQUIRED FOR COMPLETE HEMOLYSIS OF RED CELLS IN STANDARD SAPONIN SYSTEMS

NAME	AGE	SUBJECT	ASSOCIATED CONDITIONS	HEMATOCRIT	R.B.C.	TIME FOR COMPLETE HEMOLYSIS BY SAPONIN
						<i>seconds</i>
B. B.	20	Normal	None	44	4,900,000	330
R. P.	32	Normal	None	48	5,100,000	300
B. T.	40	Normal	None	38	4,600,000	320
C. C. E.	26	Normal	None	44	4,800,000	310
R. J.	20	Normal	None	38	4,500,000	350
M. F.	22	Normal	None	39	4,700,000	350
F. H.	28	Normal	None	44	5,000,000	210
C. E.	26	Normal	None	42	4,900,000	330
H. D.	31	Normal	None	34	4,000,000	360
O. B.	30	Normal	None	45	5,100,000	290
R. C.	38	Nutritional anemia	Addison's disease	32	3,200,000	380
E. R.	40	Pernicious anemia		34	3,600,000	310
F. S.	50	Pernicious anemia		40	4,100,000	330
A. S.	28	Hemolytic jaundice		40	3,800,000	280
R. D.	46	Secondary anemia	Carcinoma of the bowel	30	3,400,000	310
N. C.	30	Post-operative bleeding	Intussusception	34	3,200,000	310

Galvanometer readings were taken every ten seconds, a stop watch being used for this purpose. As each galvanometer reading represents an actual cell count, the percentage of cells present at any given time could be determined and the percentage of hemolysis readily estimated.

All experiments were carried out at a constant temperature of 24° to 26°C.

*Experimental*

Eight carefully studied patients showing various degrees and types of nephritis were chosen for our investigations. The salient features of each case and

CHART I. HAEMOLYSIS OF RED CELLS FROM NEPHRITIC AND NON-NEPHRITIC PATIENTS BY .002 GM. SAPONIN

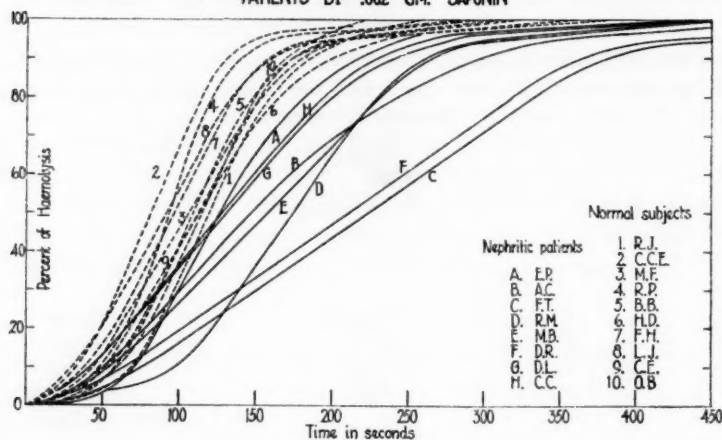
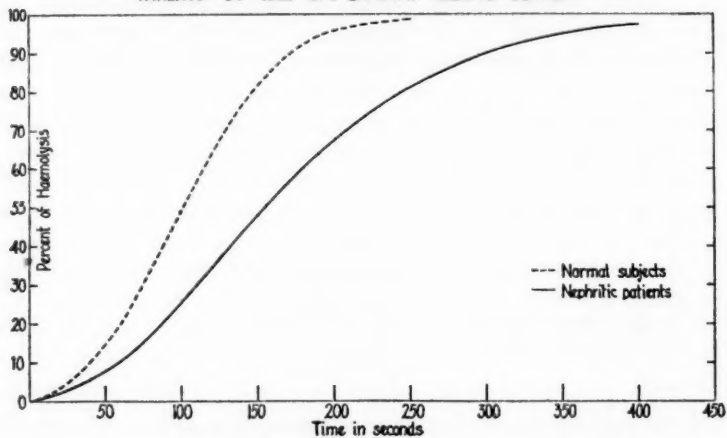


CHART II. HAEMOLYSIS OF RED CELLS FROM NEPHRITIC AND NON-NEPHRITIC PATIENTS BY .002 GM. SAPONIN. AVERAGE CURVES



the time for complete hemolysis of their red cells in a saponin system is presented in table 1.

As controls, ten normal subjects and six patients with various types of anemia, in whom there were no evidences of nephritis, were chosen. The

time required for complete hemolysis of their red cells in a saponin system is shown in table 2.

On comparing the times required for complete hemolysis in the foregoing tables it is at once seen that the red cells of nephritic patients hemolyze more slowly than those of normal subjects and of patients with various types of anemia. This phenomenon is more clearly illustrated in chart 1, where the velocity of hemolysis by saponin of blood from normal subjects and nephritic patients is presented. The curves obtained from hemolysis of cells from nephritic patients show a greater variation in range than those obtained in normal subjects. The range of each group, however, is fairly distinct. This fact is better demonstrated in chart 2, where the curves represent the average points (percentage of hemolysis against time) of both groups.

From the above tables and charts it is obvious that the red cells of nephritic patients are more resistant to saponin hemolysis than those of the control group. Believing that this might be due to the adsorption on the cell interspaces of some protective substance, we attempted to remove this substance by repeated washings of red cells. At various times we subjected red cells from nephritic patients to carefully controlled washing with normal saline for three hours, and then submitted them to saponin hemolysis, precisely as in the previous experiments. Following this procedure, the time required for complete hemolysis was reduced to figures which were within the limits of normal. These results appeared to point to the presence of some protective substance in the serum of nephritic patients. With this in view, we proceeded to investigate the effect on saponin hemolysis of serum from normal and nephritic groups.

#### B. EFFECT ON HEMOLYSIS OF SERUM FROM NEPHRITIC PATIENTS

Red cell suspensions were made up according to the technique described above, the same apparatus being used.

##### *Method*

As serum is to be added to the cell suspension, the volume of red cells in saline was made up in the Evelyn tube to 17.5 cc. instead of 19 cc. as before. The reason for this was that 0.5 cc. of serum was added to the suspension as well as 2.0 cc. of saponin solution, making a total volume of 20 cc. As Ponder (9) has shown, normal serum has an inhibitory effect on hemolysis by serum, so that a saponin dilution of 1:600 (0.002 gram saponin) made up to a volume of 20 cc. of cell suspension containing 0.5 cc. of serum would produce no hemolysis. Consequently, we were obliged to use more saponin. For our purposes it was found convenient, after various trials with other dilutions, to use 1 cc. of 1:500 and 1 cc. of 1:100 dilution of saponin, giving a total of 0.012 gram of saponin in the system, as compared with 0.002 gram in the previous systems where we dealt with red cells without serum. The final solution, therefore,

contained, when the hematocrit was 45, 2 cc. of red cell suspension, 15.5 cc. of 0.85 per cent saline, 0.5 cc. of serum, 1.0 cc. saponin 1:500, and 1.0 cc. of saponin 1:100; giving a total volume of 20 cc. Readings of the galvanometer were taken at intervals of ten seconds or of one minute, according to the rate of hemolysis.

#### EXPERIMENTAL

Four cases of chronic nephritis with anemia were investigated. These same cases had been used in the previous experiments with simple saponin systems (F. T., R. M., E. P., and D. L.). As controls, four normal subjects were used (C. E., R. P., L. T., and O. B.). Four cases, presenting anemia of various types and showing us evidences of nephritis (F. S., R. D., E. R., and R. C.) were also investigated to ascertain whether any effects obtained were restricted to the group of nephritic patients with anemia and did not apply to cases of anemia not nephritic in origin.

A series of experiments were then carried out as follows:

*Experiment I.* A saline suspension of cells from normal subjects was placed in contact with 0.5 cc. of each subjects' homologous serum for two minutes and saponin added. The length of time for almost complete hemolysis is presented in table 3. It will be seen from the above table that the time for complete hemolysis varies from two minutes and forty seconds (160 seconds) to fifteen minutes (900 seconds), with an average of approximately eight minutes. In two cases, C. E. and O. B., the time required for complete hemolysis is less where serum is added than when only washed cells are used (table 2). It should be noted that the times are not strictly comparable, however, as more saponin is used in the experiments where serum is used. These results can be directly compared with those which follow in tables 4, 5, 6, and 7. The velocity of hemolysis is shown in chart 3.

*Experiment II.* Red cells from nephritic patients were placed in contact with 0.5 cc. serum from normal subjects in exactly the same manner as in experiment I. The results are shown in table 4 and chart 3.

In this case, conditions were the same as in experiment I, normal serum being used, but here red cells from nephritic patients were subjected to serum instead of normal red cells. The time for hemolysis is essentially the same as in experiment I, the extremes being two minutes and five seconds (125 seconds) and twenty minutes (1200 seconds), and the average twelve minutes as compared with the average of eight minutes in experiment I. The same sera, C. E., R. P., O. B. and L. T. were, of course, chosen as in experiment I.

*Experiment III.* In this experiment red cells from nephritic patients were placed in contact with 0.5 cc. of their own homologous serum. The findings are shown in table 5 and chart 3.

In experiments I and II normal serum was used; in experiments III and IV, serum from nephritic patients. The time for hemolysis as shown in table 5, varied from forty minutes to 139 minutes, with an average of eighty minutes.

TABLE 3  
CELLS FROM NORMAL SUBJECTS PLUS NORMAL SERUM

RED CELLS 0.1 CC. BLOOD	SERUM 0.5 CC.	SAPONIN IN 20 CC. VOL. SYSTEM	TIME FOR COMPLETE HEMOLYSIS	
		grams	minutes	seconds
C. E.	C. E.	0.012	2	40
R. P.	R. P.	0.012	10	6
O. B.	O. B.	0.012	3	40
L. T.	L. T.	0.012	15	

Mean time for complete hemolysis = 8 minutes.

TABLE 4  
CELLS FROM NEPHRITIC PATIENTS PLUS NORMAL SERUM

RED CELLS 0.1 CC.	SERUM 0.5 CC.	SAPONIN IN 20 CC. VOL. SYSTEM	TIME FOR COMPLETE HEMOLYSIS	
		grams	minutes	seconds
E. P.	C. E.	0.012	2	5
F. T.	R. P.	0.012	20	
R. M.	O. B.	0.012	15	
D. L.	L. T.	0.012	10	40

Mean time for complete hemolysis = (12 minutes).

TABLE 5  
CELLS FROM NEPHRITIC PATIENTS PLUS SERUM FROM THE SAME PATIENTS

RED CELLS 0.1 CC. BLOOD	SERUM 0.5 CC.	SAPONIN IN 20 CC. VOL. SYSTEM	TIME FOR COMPLETE HEMOLYSIS
		grams	minutes
E. P.	E. P.	0.012	40
F. T.	F. T.	0.012	139
R. M.	R. M.	0.012	76.5
D. L.	D. L.	0.012	64

Mean time for complete hemolysis = 80 minutes.

*Experiment IV.* Red cells from normal subjects were placed in contact with 0.5 cc. serum from nephritic patients exactly as in the previous experiments. The results are shown in table 6 and chart 3.

Again serum from nephritic patients was used, as in experiment III, but the cell suspension in this case contained normal red cells. It will be seen from

table 6 that the range of time for hemolysis lay between thirty-five minutes and 120 minutes, with an average of eighty-two minutes. This compares well with the average time of eighty minutes in experiment III. In experiments III and IV the same sera, E. P., F. T., R. M. and D. L. were chosen.

*Experiment V.* This experiment was conducted along the same lines as the foregoing, but here cells from patients with an anemia, not associated with nephritis, were placed in contact with 0.5 cc. of each patient's homologous serum in saline suspension. The results are presented in table 7.

A series of experiments was carried out conforming precisely to conditions in experiments I to IV, save that nephritic cells and serum were replaced by

TABLE 6  
NORMAL CELLS PLUS SERUM FROM NEPHRITIC PATIENTS

RED CELLS 0.1 CC. BLOOD	SERUM 0.5 CC.	SAPONIN IN 20 CC. VOL. SYSTEM	TIME FOR COMPLETE HEMOLYSIS
		grams	minutes
C. E.	E. P.	0.012	68
R. P.	F. T.	0.012	104
O. B.	R. M.	0.012	35
L. T.	D. L.	0.012	120

Mean time for complete hemolysis = 82 minutes.

TABLE 7  
RED CELLS FROM ANEMIC PATIENTS PLUS SERUM FROM THE SAME PATIENTS

RED CELLS 0.1 CC. BLOOD	SERUM 0.5 CC.	SAPONIN IN 20 CC. VOL. SYSTEM	TIME FOR COMPLETE HEMOLYSIS	
		grams	minutes	seconds
R. C.	R. C.	0.012	5	5
E. R.	E. R.	0.012	7	8
F. S.	F. S.	0.012	4	20
R. D.	R. D.	0.012	4	

Mean time for complete hemolysis = 6.5 minutes.

cells and serum from non-nephritic anemic patients. The results of this set of experiments demonstrate that the serum of these anemic patients, compared with the serum of normal subjects, shows no increased inhibition of saponin hemolysis. The findings in table 7 illustrate this point, showing the average time for hemolysis to be five minutes eight seconds, with a range of four minutes to seven minutes eight seconds. Consequently, for purposes of simplicity, the other pertinent data of these experiments are not presented in table form, nor in charts 3 and 4.

Chart 4 is a replica of chart 3, the two curves showing the average points of all curves in the case of the normal and the nephritic group respectively.



From the foregoing tables and charts it is readily seen that there is a striking difference between the inhibitory effect of serum from nephritic patients and that of serum from normal subjects. In experiments III and IV, where sera from the nephritic group were used, the average time for hemolysis was in one

CHART III. CURVES ILLUSTRATING CROSS EXPERIMENTS WITH RED CELLS AND SERUM FROM NORMAL SUBJECTS AND NEPHRITIC PATIENTS. SAPONIN HAEMOLYSIS.

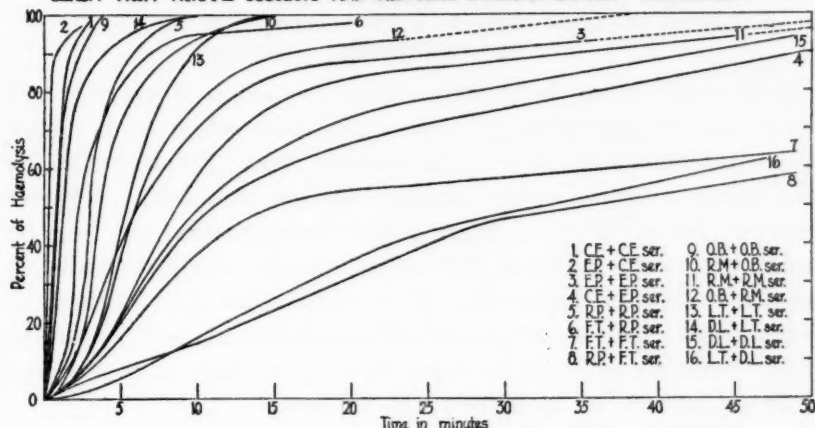
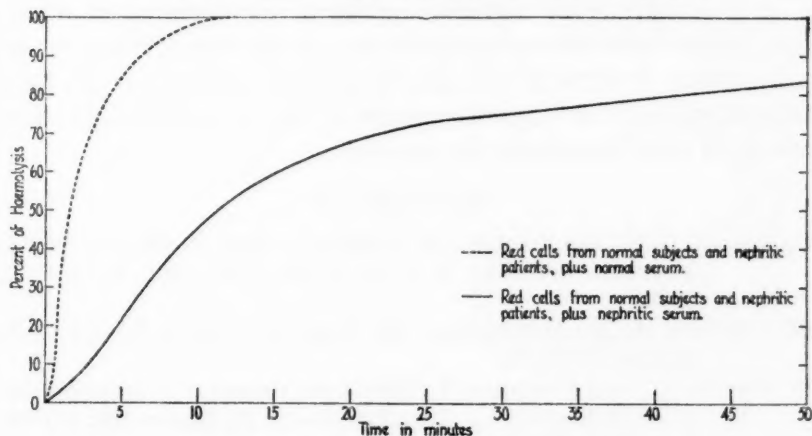


CHART IV. AVERAGE CURVES FROM CHART III. SAPONIN HAEMOLYSIS



case eighty minutes, and in the other eighty-two minutes; whereas in experiments I and II, where normal sera were involved, complete hemolysis occurred in an average time of eight minutes in one experiment and twelve minutes in the second. Thus, taking an average of all cases, any group of cells when in

contact with serum from a nephritic patient requires seventy minutes longer for hemolysis than when in contact with normal serum.

It will be noted in reference to the charts that in some cases the inhibitory effect of serum from nephritic patients was more marked when it was placed in contact with cells from normal subjects than when added to its own homologous cells. The explanation for this finding is obscure, but it may be assumed that heterologous cell-serum complexes play a rôle.

#### DISCUSSION

Our observations have shown that the serum of patients with glomerulo-nephritis has a marked inhibitory effect on hemolysis by saponin. This view is in keeping with Ponder<sup>9</sup> who has shown that normal serum inhibits saponin hemolysis. The reason for this evades us. Work is now being carried out to determine the relationships of the various lipoids and proteins in the serum to this phenomenon. It is conceivable that if there are substances which affect the red cell membrane in any way altering their reaction to saponin these substances may play a rôle in influencing red cell metabolism in disease.

#### CONCLUSIONS

1. Red cells from nephritic patients hemolyze more slowly than those from normal subjects in saponin hemolytic systems.
2. Serum from nephritic patients, when added to red cells of normal subjects or nephritic patients, has a marked inhibitory effect on their hemolysis by saponin.

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## INTRACRANIAL ARTERIAL ANEURYSMS\*

NORBERT ENZER† AND EDWARD D. SCHWADE

*Case 1.* A young white male, 30 years of age, whose past medical history was entirely irrelevant to the present condition, was hospitalized in a state of extreme nervousness, complaining of frontal headache, nausea, and vomiting. On the evening prior to the day of admission, while fixing a radio aerial on the roof of his home, he suddenly became dizzy and apparently was unconscious for about fifteen minutes. When he finally recovered consciousness, he was able to make his way to his apartment downstairs, where he complained of a right-sided pounding headache and a sense of exhaustion. His wife noticed him to be extremely pale. Within the next hour he vomited several times. Late that night he had a chill, or at least felt chilly. Within a few hours after admission to the hospital he became semi-stuporous. His rectal temperature was 100 degrees; pulse 84 per minute; blood pressure 160/90. Physical examination failed to reveal any evidence of trauma, and the entire physical examination did not disclose any abnormalities. A tentative diagnosis of an acute gastrointestinal disturbance was made. The eyegrounds were clear. There was no evidence of cranial nerve palsies, and all reflexes were of normal activity. The pupils were regular and equal and reacted smartly to light. The following day he became irrational. His respirations were labored. The blood pressure was now 142/76, pulse 70, temperature 99.4 degrees, and respirations 22. He vomited frequently. There appeared to be signs of meningeal irritation, since he now complained of pain and stiffness in the neck. Kernig's sign was not present. On the following day there was extreme restlessness, irritability, and periods of disorientation and irrationalism. Some stiffness of the neck was still present. During this day the blood pressure ranged from 120 to 140 systolic and 60 diastolic. The rectal temperature ranged just below 100 degrees. On the third day after admission to the hospital, he was somewhat more rational and coherent, complaining of stiffness of the neck. The left pupil was now noted to be slightly larger than the right and reacted better to light. No abnormal reflexes were observed but normal reflexes were not obtained. Biceps and triceps reflexes were diminished. Areas of anesthesia or paresthesia were not found. On the fourth day after admission to the hospital, the right pupil was definitely smaller than the left, and there was a paralysis of the right external rectus muscle. He now complained of some diplopia. Fundus exam-

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† Director of Laboratories, Mount Sinai Hospital, Milwaukee.

ination revealed blurring of both disks and dilated veins. There was not any evidence of retinal hemorrhage. On this day a diagnosis of increased intracranial pressure was made, and the condition seemed to attending physicians to be an acute inflammatory process. A spinal puncture done on the fourth day after admission released bloody fluid which, upon settling, was xanthochromic. The spinal fluid R.B.C. count was 60,000, W.B.C. 800, differential: polys 91 per cent, lymphocytes 9 per cent. All cultures were sterile. Two days later the spinal fluid was still bloody, with 40,000 R.B.C., 685 W.B.C., 90 per cent polys, and 10 per cent lymphocytes, and still sterile. Both spinal fluid and blood Wassermans were negative. From then on almost daily spinal and cisternal punctures were made. Both the amount of blood in the spinal fluid and the spinal fluid pressure progressively diminished. Consultants now considered this to be some type of traumatic intracranial lesion. Symptoms persisted chiefly in the form of headache, irritability, periods of stupor, and disorientation which were usually relieved after spinal puncture. On the sixth day after admission the sixth cranial nerve was palsied, the neck was stiff, and Kernig's sign was present for the first time. All tendon reflexes were sluggish. Pain on the right side of the head became more marked, and finally, ten days after admission, there was a complete paralysis of the left side of the body, and the patient was entirely irrational. Thinking that the lesion was a hemorrhage into the subdural space, a decompression was performed eleven days after admission to the hospital, following which the patient developed hyperpyrexia and edema of the lungs. Death occurred two days after the operation. One consultant who saw the patient on the sixth day after admission to the hospital considered the condition to be a meningeal syndrome due to spontaneous subarachnoid hemorrhage, probably the result of the rupture of a congenital cerebral aneurysm.

Blood counts taken almost daily revealed a leukocytosis ranging from 15,000 to 18,000, always with a relative polymorphonuclear leukocytosis of from 80 to 90 per cent. Daily urinalyses disclosed a slight to moderate albuminuria.

The post mortem examination afforded positive findings only in the brain. Other organs did not contribute to the pertinent findings except that it is important to state that the heart and aorta were free of evidences of mural thrombosis and inflammatory lesions, acute or chronic. The lungs were quite free of any changes except terminal edema. Examination of the head disclosed the recent right temporal decompression. The remainder of the skull was free of any evidences of abnormalities, traumatic or developmental. The dura over the left temporal and parietal areas was slightly thicker than elsewhere. The subarachnoid space was free of fluid and the cortex rather dry. In the visible portion of the right parietal lobe in situ, there was an area of yellow discoloration about 5 cm. in diameter. Convolutions were somewhat flattened and capillaries were engorged. The temporal lobe was firmly adherent to the dura on the ventral surface. Around the base of the brain there was an excess of clear

fluid. The brain weighed 1500 grams. Along the posterior surface of the right temporal lobe there was hemorrhagic infiltration of the meninges and the adjacent brain tissue, which in turn exhibited a yellow discoloration such as might have resulted from old blood pigment. The diameter of the entire area was 3 cm. Yellow pigmentation was present along the sheaths of the optic nerves and chiasm. Along the brain stem the pia arachnoid appeared thickened. Small pool-like collections of blood-stained fluid were present in the subarachnoid space at the base of the brain. The markings of the foramen magnum on the cerebellum were accentuated. The lateral aspect of the parietal and temporal lobes on the right side were softened, and the brain tissue had a soft gelatinous, edematous appearance. The right lateral ventricle was compressed, and the left dilated. Blood was not present in the ventricular system. Careful dissection of the area of hemorrhage of the right temporal lobe disclosed that the main hemorrhagic area was about 1.5 cm. in diameter. Here the brain tissue was definitely infarcted. Passing through the meninges and into the brain tissue at this point was a small artery which, when carefully separated, revealed a mass of organized blood clot protruding through the wall of the vessel forming a bud 7 to 8 mm. in diameter. Carefully separating the adherent blood clot, it was apparent that the vessel wall bulged to form a small sac through which blood had escaped. Stripping the brain of its vessels failed to reveal any other grossly discernible aneurysmal dilatations, nor was there any evidence grossly of arteriosclerosis or abnormal thickenings in the course of the vessels. The distribution from and the formation of the circle of Willis appeared to be normal. No congenital defects were noted. The microscopic examination of the cerebral vessels, including the area of aneurysm, revealed a complete thrombosis of the aneurysm with considerable intimal hypertrophy at the mouth of the aneurysm, but without evidence of cholesterol or calcium deposit. At the origin of the aneurysm the elastic lamina was split and frayed and disappeared into the thinned-out wall of the sac. The media similarly disappeared to form a thin condensed fibrous sac which ultimately lost itself in the hemorrhagic mass. Section of other portions of the brain did reveal small out-pouchings in the arteries of the subarachnoid space. Two were serially sectioned and proved to be true aneurysmal dilatations occurring generally adjacent to but not at the bifurcation of the vessel. The centrum of the affected area was necrotic and rather massively hemorrhagic. The local disturbance of the arterial circulation was reflected in the relatively wide zone of edema and petechial hemorrhages surrounding the central zone of necrosis.

Diagnosis: Thrombosis of aneurysm of cerebral artery and infarction of right temporal lobe.

*Case 2.* A further instance of this type of lesion was encountered at post mortem examination of a young man 23 years of age who had been found dead. The circumstances did not indicate violence. The base of the brain was bathed



in hemorrhage which had its origin in a ruptured aneurysm of the anterior communicating artery. The aneurysm was approximately 5 mm. in diameter.

Diagnosis: Ruptured intracranial aneurysm.

*Case 3.* Similarly, a woman 40 years of age suddenly complained of severe headache, dizziness, and soon exhibited evidence of left facial paralysis. Within the day, the left eye became painful, and coma was well-developed within 24 hours after the initial symptom. Bloody fluid was obtained by spinal puncture. Death occurred in 48 hours. An aneurysm located at the origin of the left middle cerebral artery had ruptured, and the base of the brain was bathed in blood. The aneurysm was approximately 6 mm. in diameter.

Diagnosis: Ruptured cerebral aneurysm.

Five instances have been encountered clinically; that is to say, the diagnoses have not been verified by post mortem investigation. In all instances the diagnosis was based upon evidence of a mild hemorrhage into the spinal fluid and symptoms of headache, dizziness, and a history of previous similar episodes. In these experiences, all other plausible explanations for the hemorrhage were absent. In effect, there was not any evidence of trauma, infection, alcoholism, cardiorenal disease, or syphilis, and the investigation did not disclose evidence of other types of intracranial pathology. Two of these patients were men, aged 43 and 45 years, and three were women, aged 42, 45, and 52 years. While these diagnoses lack substantial proof, they are mentioned here as indicating the possibility of diagnosing this condition during life, and also to emphasize the type of case associated with intermittent hemorrhage. One of these cases may be reported in more detail. Mrs. M. W., aged 45, complained of headache, pounding at first, later more constricting. The onset was sudden following a similar but milder attack six weeks previously. The spinal fluid was bloody, and by repeated tapplings a progressive diminution in the hemorrhage was followed, until finally the spinal fluid was xanthochromic only. The improvement in the character of the fluid paralleled progressive diminution in the severity of the symptoms. Objectively there was found only inequality of the pupils which was present for the first few days, finally disappearing completely. In this instance,

too, there was lack of evidence of other likely causes of spontaneous hemorrhage into the subarachnoid space.

Common to these five cases was a slight fever, mild leukocytosis with relative polymorphonuclear leukocytosis, albuminuria, and absence of hypertension or cardiorenal disease. All complained of headache, and all for a few hours shortly after the onset of the condition complained of slight pain in the back of the neck and stiffness of the neck.

Our experience with this condition is limited then to three instances encountered at post mortem and five cases which seem to us deservedly to belong in this diagnostic category. Our post mortem experience is much lower than has been reported in the literature, since we have found it only three times in approximately 2500 necropsies. Fearnside<sup>1</sup> found aneurysm 44 times in 5,432 necropsies. Osler<sup>2</sup> had demonstrated the condition 12 times in 800 necropsies. Pitt<sup>3</sup> and Conway<sup>4</sup> each found 23 cases in 9,000 and 6,632 necropsies respectively. Of 11,500 necropsies at the Institute for Legal Medicine in Vienna, 157 deaths were due to ruptured aneurysm (Szekely<sup>5</sup>). Busse<sup>6</sup>, by meticulous dissection of the cerebral vessels, found 39 aneurysms in 400 autopsies. Numerous other investigators report varying experiences so that, in all likelihood, the correct incidence of this condition is yet to be established, and perhaps the point is not of great importance, since there is at least ample proof in the literature that the condition is of sufficient frequency so that it should be remembered by clinicians and pathologists alike.

To clinicians and pathologists who may be involved in medicolegal controversy, a knowledge of this condition is of great practical importance. The occurrence of the clinical syndrome with recovery may be closely related in point of time with trauma or strain. Similarly, sudden death from massive hemorrhage may occur during employment or at a time coincident with trauma or great physical stress. In such cases, the attending pathologist must be careful and painstaking in his dissection since the aneurysm may be small and difficult to find. It is not at all unlikely that hemorrhages into the subarachnoid and even subdural space have been incorrectly attributed to violence.

Stumpff<sup>7</sup> 100 years ago reported 13 cases collected from the literature. In 1887, Eppinger<sup>8</sup> made his extremely important contribution concerning the congenital nature of the lesion. Isolated reports appeared from time to time in the 19th century, among the most important of which was the contribution in 1894 by Von Hoffman<sup>9</sup>, whose analysis of 78 cases emphasized the importance of intracranial aneurysm as a cause for sudden death. Sir William Gull<sup>10</sup> in 1859 emphasized the clinical frequency and Gowers<sup>11</sup> in 1893 described the anatomical distribution in 154 cases. Among others who reported this condition in the 19th century are Hodgson<sup>12</sup> and Lebert.<sup>13</sup> In the twentieth century important contributions to the literature have been made by Fearnside<sup>1</sup>, Turnbull<sup>14</sup>, Forbus<sup>15</sup>, Schmidt<sup>16</sup>, Tuthill<sup>17</sup>, Szekely<sup>5</sup>, Symonds<sup>18</sup>, Parker<sup>19</sup>, Pawlowski<sup>20</sup>, Beadles<sup>21</sup>, and many others.

The etiology of these aneurysms has been variously ascribed to (1) infection, (2) arteriosclerosis, (3) congenital defects, (4) trauma, (5) embolism, and (6) syphilis. Eppinger's congenital theory has received general support particularly from Fearnside<sup>1</sup> and Turnbull<sup>14</sup>, and more recently Forbus<sup>15</sup>, other authors in general adopting the explanations of these investigators. Chief support of the congenital theory is derived from the following points: (1) the aneurysms are frequently multiple; (2) they most frequently occur at points of bifurcation; (3) they are found chiefly in young individuals; (4) there is generally absence of evidence of inflammatory reaction in the vessel involved; (5) the absence of systemic arteriosclerosis or even arteriosclerosis of cerebral vessels. Study of the aneurysms discloses an apparent defect in the muscularis although controversy has waged between two schools: those who believe the primary defect to have been absence of the elastic tissue, and those who attribute the defect to an absence of muscularis (Forbus<sup>15</sup>).

In the three cases available to us for study, the muscularis at the margins of the aneurysm sac faded completely into the hemorrhagic mass. The wall of the sac which could be identified as such did not have in any of the three cases reported here remnants of smooth muscle fibers. Elastic tissue fibrils were

massed at the margins of the aneurysm and spread in a frayed fashion over and through the aneurysm sac wall. All sections disclosed an intimal sclerosis characterized by piling of an edematous collagenous type of thickened intima in which we were not able to demonstrate lipoid deposit. Two of the instances reported here occurred at the bifurcation of the vessel. One could not be proven as occurring at the bifurcation, but seemed to take origin directly from an otherwise intact vessel wall.

Tuthill<sup>17</sup> reported six instances of intracranial aneurysm. In an attempt to account for medial defects, Tuthill was able to establish that these defects occurred frequently at bifurcations, but believed that artefacts of embedding accounted for many of these irregularities, and on the basis of elastic tissue changes and evidences of sclerosis attributed the aneurysm to arteriosclerotic degeneration. He concludes that neither the character of the wall of the aneurysm, the presence or absence of intimal changes, nor the size, location and number are adequate criteria to determine whether or not these aneurysms are congenital or arteriosclerotic in origin.

Reuterwall<sup>22</sup> claims to have found evidences of healed lacerations which he attributed to the results of trauma to the vessel wall as the result of repeated irritations by contact with the skull. Pawlowski<sup>20</sup> emphasizes the importance of trauma and reports three out of nine deaths following injury. Harbitz<sup>23</sup> calls attention, too, to the influence of trauma. Jungmeichel<sup>24</sup> reports 19 cases from the literature in which aneurysm ruptured following cranial injury in which no other causes for aneurysm were found.

Syphilis seems not to be an important factor. The tendency for this infection to produce endarteritis seems to preclude the formation of aneurysm in small vessels.

Mycotic aneurysm from infection and embolism is of frequent occurrence but will not be discussed here.

An important indirect evidence of the congenital nature of the lesion is afforded by the frequency of other types of congenital anomalies in the vessels of the circle of Willis and its branches. The association of cerebral aneurysm with coarctation

of the aorta is additional evidence of the congenital nature, and this has been commented upon by Hamilton and Abbott<sup>25</sup> and Waltman and Sheldon.<sup>26</sup>

The anatomical distribution has been tabulated by Szekely<sup>5</sup>, Gowers<sup>11</sup>, Lebert<sup>13</sup>, and others whose articles should be consulted for more statistical data. The age is predominantly under 50 with the majority under 35 years. Children under 10 years of age are infrequently affected. The incidence among females is slightly higher than among males.

The signs and symptoms of cerebral aneurysm vary with the type of aneurysm and with its behavior. Of the three main types, the arteriosclerotic form generally occurs in individuals beyond the age of 40. The congenital form, which in effect may ultimately be included in the arteriosclerotic form is apt to occur under the age of 35. The embolic type is related to endocarditis or other forms of sepsis. The less common forms, traumatic (?) and syphilitic, are not necessarily of characteristic age distribution.

The clinical features may be divided into two main groups: those pertaining to the aneurysm before rupture, and those associated with rupture. The latter group may be subdivided into (a) massive hemorrhage with death, and (b) intermittent hemorrhage. Prior to actual hemorrhage, the signs and symptoms are those of pressure, and may or may not be associated with localizing signs. These are dependent upon the location and size of the aneurysm. The symptoms may simulate those of brain tumor. On the other hand the increasing growth of the aneurysm may cause local anemia and infarction of brain tissue. Headache and localizing throbbing pain are of frequent occurrence. Thrombosis of the aneurysm may be the explanation of symptoms resulting from localized infarction. Case 1 is an illustration of such an episode. Important is it to remember, therefore, that thrombosis of the aneurysm in contrast to rupture of the aneurysm may lead to disastrous results. The thrombosis of the aneurysm may follow upon hemorrhage, so that the clinical course of events will indicate the onset with hemorrhage, and the chain of symptoms following result from thrombosis.



Therefore, in spite of progressive improvement in the character of the spinal fluid with respect to the hemorrhage, the clinical course may be downhill because of the development of an occlusion of the aneurysm.

When hemorrhage does occur, the onset is usually sudden and may be associated with mild or severe exertion or trauma. History of headache or pain in the head, sometimes attacks of nausea and vomiting, have been described following sneezing and coughing spells. Conditions which will raise the intracranial arterial pressure may produce a transient chain of symptoms. The seepage of blood may be small or large. If small, there may or may not be a loss of consciousness. Sometimes, transient loss of consciousness is the only symptom. Repeated attacks may occur over a period of years. Fearnside<sup>1</sup> cites a case covering a lapse of 20 years. If the hemorrhage is great, a fatal result occurs in a few hours. There is loss of consciousness, dilatation of the pupils, cyanosis and stertorous breathing. The pulse at first slow, later becomes rapid. Localizing signs do not appear until the patient survives the initial period of coma. Convulsive episodes may accompany the attack followed by hemiplegia or other local neurological features. Fever and leukocytosis are fairly constant findings. Interesting is the occurrence of a transient albuminuria which probably is derived through the same mechanism as the albuminuria of cranial injury. Spinal fluid is increased in amount and pressure, contains blood in varying amounts, and depending upon the duration of the attack, becomes xanthochromic. For further detail concerning the clinical features, the article by Parker<sup>21</sup> is comprehensive.

It is interesting here to note that vomiting is a frequent symptom at the onset of the episode, and an initial diagnosis of a gastroenteric disturbance is frequently made. This obtained in the first case reported here.

In the first case reported here the aneurysm, so far as all gross anatomical findings are concerned, was solitary. Accidental microscopic findings, however, disclosed apparently two other aneurysms: one a fusiformly dilated vessel filled with thrombus



and located between two gyri. Serial section of this block of tissue indicates it to have been a fusiform dilatation. The size of the vessel would seem to bear this out. Likewise, lying free in the subarachnoid space, a smaller vessel was found containing a fragment of thrombus and presenting a localized sacculation. Serial section of this block proved it to be a true aneurysm and not an artefact or a point of branching. It is to be noted that in these two lesions there was not any evidence of arteriosclerosis. The defect was entirely that of media, although elastic tissue stains showed very few elastic fibrils in the sac. The most important feature of the large aneurysm was the obvious sclerotic thickening at its margins. The thickening involved the intima only. The afferent and efferent vessels of the main aneurysm did not exhibit any arteriosclerosis. Taken all in all, the character of the thrombosed aneurysm and the accidental finding of the microscopic aneurysms and the absence of primary arteriosclerosis, there seems to be reasonable evidence in this case to conclude that these aneurysms were congenital in origin. The histological findings in Cases 2 and 3 were limited entirely to the aneurysms, and may be briefly stated<sup>7</sup> as similar to those in Case 1; namely, a localized thickening of the margin of the aneurysm without evidence of sclerosis in adjacent vessels. The aneurysms in these cases were solitary, and in view of the fact that there was not any evidence of generalized or even patchy cerebral arteriosclerosis and that the post mortems did not disclose any evidences of endocarditis or mural thrombosis of the heart or aorta, and because of the location of these aneurysms at points of bifurcations of the vessels, it is likely that they can be included among those of congenital origin.

#### SUMMARY

The post mortem findings of three cases of ruptured aneurysm are described. Clinical records of five additional cases have been included. The congenital theory of the origin of these aneurysms seems to be most favored, and more adequately explains the three instances reported here. The clinical features have been stressed and the importance of this condition in forensic medicine

emphasized. As a clinical entity it should be borne in mind since it is likely that a correct clinical diagnosis can be arrived at. It should be remembered in all instances of obscure or otherwise unaccountable hemorrhage into the subarachnoid space.

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## SOME POSSIBLE EFFECTS OF NURSING ON THE MAMMARY GLAND TUMOR INCIDENCE IN MICE\*

JOHN J. BITTNER

*Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine*

This communication considers in more detail the change in the incidence of mammary gland tumors in breeding females following the foster-nursing of progeny from a high breast tumor line by mothers from a relatively low tumor stock.

### STOCKS OF MICE

#### *"A" Stock*

This strain of albino mice had been inbred for forty generations by brother-to-sister matings. More than 80 per cent of the breeding females surviving 6 months or longer developed spontaneous carcinoma of the mammary gland (Bittner, 1935). The average cancer age was  $11.5 \pm 0.1$  months.

#### *CBA or "X" Stock*

Representatives of this strain were obtained from Dr. L. C. Strong (1936). They had been inbred for approximately 25 generations. Observation on 125 breeding females gave a breast tumor incidence of 13.5 per cent at an average age of 21 months. In addition, 8.7 per cent developed primary lung carcinoma, average age 28 months, and 15.9 per cent succumbed to other types of tumors, predominately sarcomas of various types.

New-born young of the A stock were removed from their mothers and fostered by females of the CBA stock until weaning age. The females were mated to their brothers and were continued as breeders until the end of their cycle. All the progeny were nursed by their mothers.

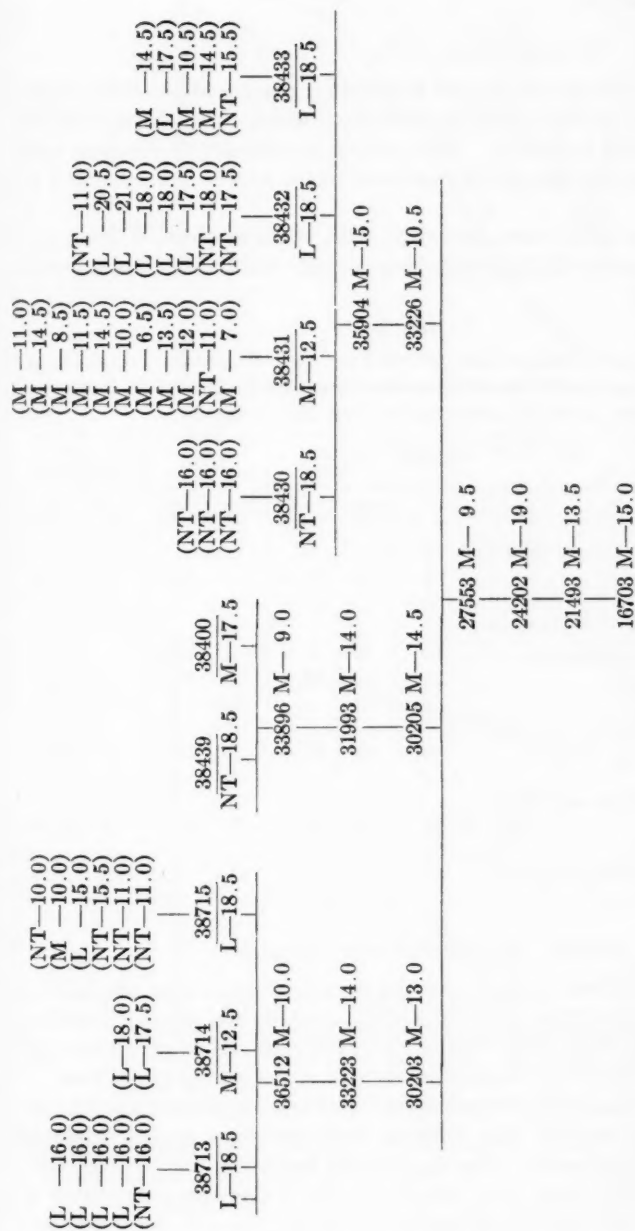
### OBSERVATIONS

#### *Control "A" Stock Breeding Females*

The number of breeding females of the A stock observed to date is 575, of which 474 or 82.4 per cent have developed mammary gland carcinoma. In addition, 15 or 2.6 per cent have given rise to bronchial lung carcinoma (table 1).

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Ten Successive Generations  
of Mammary Carcinoma

CHART 1

Pedigree of the sub-line of the A stock showing the ancestry of the breeding females which were fostered by females from the CBA stocks are designated by underlined numbers. Progeny of the fostered females are represented by brackets with the diagnosis and age at death represented in months. M = carcinoma of mammary gland; L = primary bronchial lung carcinoma; NT = non-tumor.

Included among the animals with breast cancer are some which also had primary pulmonary tumors.

*Fostered Females of the "A" Stock*

Three litters of A stock young were fostered by females of the CBA stock. The pedigree of the sub-line of the A stock from which these litters were descended is represented in chart 1. The fostered females are represented with underlined numbers with the age in months at which tumors were recorded or autopsy performed.

Of the 9 females which were nursed by CBA stock mothers, 3 developed mammary gland tumors; 4 had primary lung tumors; and 2 died non-cancerous (table 1).

TABLE 1

GIVES THE NUMBER AND PERCENTAGE OF THE FOSTERED FEMALES, THEIR PROGENY AND THE NORMAL A STOCK BREEDING FEMALES TO DEVELOP BREAST CANCER, LUNG CANCER OR TO DIE NON-TUMOROUS

	NUMBER	MAMMARY GLAND		LUNG		NON-TUMOROUS	
		num-ber	per-cent	num-ber	per-cent	num-ber	per-cent
A Stock Breeding ♀♀ Fostered by CBA ♀♀ .....	9	3	33.3	4	44.4	2	22.2
Progeny of Fostered ♀♀ Accord- ing to Diagnosis of Mothers .....	13	10	77.9	2	15.4	1	7.7
Lung Carcinoma .....	24	4	16.7	11	45.8	9	37.5
Non-Tumor .....	3	0		0		3	
Total—Progeny .....	40	14	35.0	13	32.5	13	32.5
Total—Fostered ♀♀ and Prog- eny .....	49	17	34.7	17	34.7	15	30.6
A Stock Unfostered Breeding ♀♀ .	575	474	82.4	15	2.6	86	15.0

PROGENY OF THE FOSTERED FEMALES

A few of the young from seven of the nine fostered females were also used as breeders. They were nursed by their A stock mothers and are designated in chart 1 by brackets above their mother's number with the diagnosis and age at death. Observations were secured on 40 mice of the second generation.

In table 1 the animals are grouped according to the diagnosis of their mothers. Ten of the 13 progeny from mothers with mammary tumors produced similar growths (78 per cent). The lung tumor incidence was 15.4 per cent. Fostered mothers with pulmonary tumors had 24 of their progeny mated of which 4 or 16.7 per cent had breast tumors, 11 or 45.8 per cent developed pri-



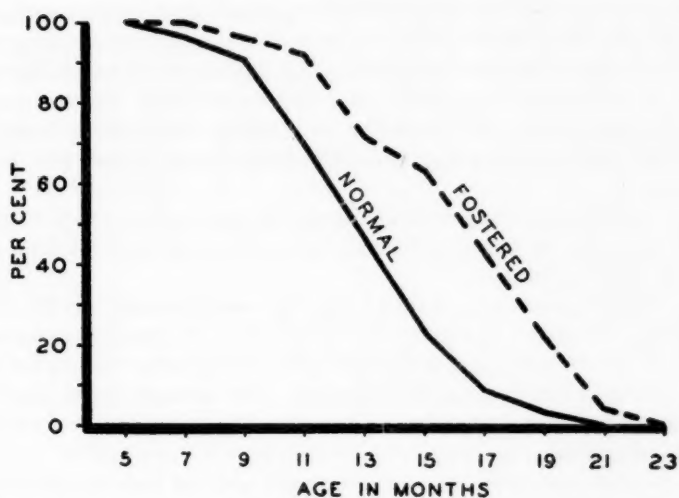


CHART 2. THE PROPORTION OF FOSTERED AND NORMAL A STOCK BREEDING FEMALES LIVING TO THE BEGINNING OF EACH AGE PERIOD OR LONGER

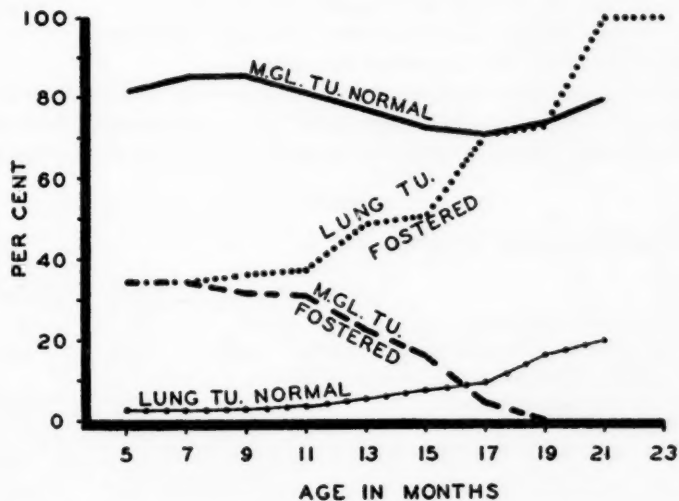


CHART 3. REPRESENTS THE PERCENTAGE OF FOSTERED AND NORMAL A STOCK FEMALES LIVING TO THE BEGINNING OF EACH AGE PERIOD OR LONGER TO DEVELOP BREAST CANCER OR PRIMARY LUNG TUMOR

mary lung tumors and the remainder (37.5 per cent) died non-tumorous. Two of the progeny included in the breast tumor class also had primary lung tumors. The three young from non-cancerous mothers likewise failed to develop tumors.

Thus, in the two generations of A stock breeding females—the first consisting of 9 fostered animals, the second of 40 progeny, we observed breast carcinoma in 34.7 per cent, lung tumors in 34.7 per cent, and 30.6 per cent died non-tumorous.

Chart 2 represents the proportion of the two groups living to the beginning of each age period or longer, and chart 3 gives the proportion developing mammary or pulmonary tumors.

The average mammary and lung tumor age was approximately the same for both groups of mice. In the fostered series, however, the non-tumorous animals lived, on the average, 3 months longer than the same class in the normal series (15.0 and 12.0 months respectively). The average age at death of all the mice of the fostered series was 14.3 months compared with 12.1 months for the control group.

The average litter size for both the control and the fostered groups of animals declined after the females had reached 261 days of age, probably showing that the animals of the two classes aged with equal rapidity as regards reproductive activity.

In addition to the A stock mice fostered by CBA mothers, another group was nursed by females of the C<sub>57</sub>B1 strain—a stock which has a mammary tumor incidence of less than one per cent (Murray and Little, 1935). In one series of 49 mice, 38 mice (83 per cent) have survived at least 13 months. Two of the mice which have died had mammary carcinoma.

Thus, it appears that the foster-nursing of mice from a stock having a high incidence of breast tumors by females from a low line reduces materially the incidence of such tumors in both the fostered animals and their progeny.

#### SUMMARY

The observations may be summarized as follows:

	<i>Normal</i>	<i>Fostered by CBA ♀</i>	<i>Fostered by C<sub>57</sub>B1 ♀</i>
Total number observed.....	575	49	47
Living to the beginning of the 14th month..	27%	65%	83%
Percentage dying before 14 months of age to develop breast cancer.....	86%	70%	25%
Total.....	82%	35%	Incom- plete

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## HEMATOLOGICAL OBSERVATIONS ON BONE MARROW OBTAINED BY STERNAL PUNCTURE\*

PETER VOGEL, LOWELL A. ERF AND NATHAN ROSENTHAL

*From the Medical Department and the Laboratories of the Mt. Sinai Hospital,  
New York, N. Y.*

Until recently, living bone marrow for diagnostic purposes was obtained only by surgical biopsy. Ghedini,<sup>9</sup> in 1908, published the first study of tibial bone marrow by biopsy and recognized its importance in the diagnosis of malaria and leishmaniasis. This procedure was subsequently employed by Peabody<sup>18</sup> and Zadek<sup>34</sup> in the various stages of pernicious anemia. As tibial marrow is usually hypoplastic, and as sternal marrow is more cellular and reflects to a greater extent the activity of bone marrow in general, Seyfarth<sup>28</sup> in 1923, suggested trepanation of the sternum for the study of bone marrow, in both sections and smears. The use of small trephines was advocated somewhat later by Tuohy and Gillespie,<sup>31</sup> Dameshek,<sup>5</sup> and Escudero and Varela.<sup>7</sup> While these procedures are minor surgical operations, the scar resulting from sternal biopsy constituted an important objection, particularly for women. A simpler method for obtaining sternal bone marrow was first described by Arinkin<sup>1</sup> in 1927 consisting of aspiration of the marrow through a spinal puncture needle. An excellent review, with a bibliography of this procedure, is presented by Segerdahl,<sup>27</sup> Nordenson<sup>17</sup> and Roversi and Tanturri<sup>25</sup> in recent monographs. In this country observations on marrow studies by sternal puncture, have been reported by Holmes and Broun,<sup>12</sup> Young and Osgood,<sup>33</sup> and Reich.<sup>21</sup>

The purpose of this paper is to evaluate the method as a

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practical routine diagnostic procedure and to report changes in the marrow during the course of various diseases, especially blood dyscrasias.

#### TECHNIQUE

Two methods have been employed in obtaining bone marrow from the sternum.

*First Method.* This technique was used in about one-half the cases. The skin over the upper sternum was prepared with iodine and alcohol, the preferable site for puncture being at the level of the upper border of the third rib in the midline of the body of the sternum. Although no anesthesia was used the puncture was only slightly painful. An 18 gauge spinal puncture needle (preferably shortened to two inches) was inserted at an angle of about  $45^\circ$ , with the point directed cephalically into the marrow cavity, entrance into which was recognized by a sudden "give" of the needle, indicating penetration of the outer table of the sternum. A tight fitting dry or (0.9 per cent saline) rinsed syringe (2 or 10 cc.) was attached to the needle and about 0.1 to 0.2 cc. of the marrow withdrawn. From this drop a white cell and megakaryocytic count was done and smears were made which were stained with a combination of Jenner and Giemsa. Occasionally, complete blood counts were taken. This technique practically avoids admixture of blood and trauma to marrow cells.

A differential count of 500 white blood cells was made. The various nucleated red blood cells were also counted, but not included in the differential count of the white cells. They were recorded in proportion to 100 white cells, thus determining the total number of leucocytes and nucleated red cells.

*Second Method.* The skin was prepared in the usual manner. Local anesthesia was injected both subcutaneously and subperiosteally. After insertion of the needle, 2 cc. of marrow fluid were withdrawn and placed in a sterile test tube containing one drop of 30 per cent sodium citrate. On withdrawal of this amount of marrow fluid, nearly all of the patients complained of a characteristic suction or vacuum pain. The method is essentially the one used by Young and Osgood,<sup>33</sup> and by Reich.<sup>21</sup>

The hemoglobin, red and white blood cell count, platelet count, and smears of the marrow fluid were done. Both supravital and plain smears were made immediately from the needle.

The citrated marrow fluid was placed in a Wintrobe tube and centrifuged for five to ten minutes. In this way a relatively accurate red cell volume, white cell volume and platelet volume was determined. Occasionally a fat layer was seen at the top of the tube. The white cell layer was carefully removed with a Wright capillary pipette, mixed to insure uniformity, and smears made. A tendency was noted for the heavy granular cells to accumulate at the bottom of this layer.

## MICROSCOPY OF BONE MARROW SMEARS

Marrow smears well stained with Jenner and Giemsa usually have the appearance of leukemic blood. The hematic marrow cells are numerous, so that 500 to 1,000 cells are easily counted. Fixed cells—such as reticulum and endothelial cells—are occasionally found. The greater number of cells may be classified arbitrarily, according to the following main groups:

1. Myeloblasts and their derivatives
2. Lymphocytes
3. Plasma cells (myelomatosis)
4. Hematogones
5. { Reticulum cells  
Endothelial cells
6. Megakaryocytes
7. Gaucher cells
8. Normal and nucleated red blood cells

The characteristics of these various cells, in smears obtained by means of the Jenner-Giemsa method, are presented herewith:

1. *Myeloblasts and Derivatives.* (a) The *myeloblast* is the most primitive cell of the myeloid series and by maturation develops into a promyelocyte, myelocyte, non-segmented (rod-shaped nucleus) and segmented neutrophile. The young myeloblasts are large cells with a finely reticulated nucleus surrounded by a rim of deep blue homogeneous cytoplasm. Two or three nucleoli may be present which frequently appear as small vacuoles through which the blue cytoplasm may be seen.

(b) *Promyelocyte.* As the myeloblast matures, the cytoplasm becomes lighter or may increase in size, but the deep bluish primitive cytoplasm may still be retained at the periphery. At this stage, azurophilic granules begin to appear throughout the cytoplasm varying from a few in one part of the cytoplasm, to many scattered diffusely over the cytoplasm and nucleus.

(c) *Myelocytes.* These cells present neutrophilic, eosinophilic, or basophilic granules which occur either at one pole or diffusely scattered throughout the cytoplasm. At this stage the nucleus assumes a more homogeneous appearance, with a disappearance of the nucleoli. The younger neutrophilic and eosinophilic granules may be more or less basophilic in appearance. As the cells approach maturity the neutrophilia and eosinophilia become more definite. Mitosis may be found in these cells as well as in myeloblasts and promyelocytes.

Variations may occur as a result of differences of maturation of the nucleus and cytoplasm. The nucleus may develop into the myelocytic type, while the cytoplasm retains its bluish color devoid of granules. The nucleus may mature still further, reaching a rod-shape or segmented form, thus appearing similar to monocytes (agranulocytic myelocytes or polynuclears)



The fragility of cells may be increased when the marrow fluid is placed in oxalate or citrate. In smears fragile promyelocytes may assume an irregular shape, resembling that of macrophages, the nucleus being of loose texture and the azure granules widely separated. These are the Ferrata hemohistioblasts. They are rarely found in smears made immediately from the aspirated bone marrow, and are not present in supravital preparations. We agree with Ringoen<sup>22</sup> that these cells are traumatized promyelocytes and myelocytes.

2. *Lymphocytes*. These are both small and large and resemble the cells in the peripheral blood.

3. *Plasma Cells*. Plasma cells are of variable size—usually larger than lymphocytes. The basophilic cytoplasm is abundant and contains an eccentric nucleus with a heavy chromatin network. A light perinuclear zone is usually present in which mitochondria are found in supravital preparations. The differentiation from erythroblasts is difficult at times. Large numbers of these cells are found in multiple myeloma.

4. *Hematogones*. These are the mystery cells of the bone marrow. They have been regarded as undifferentiated primordial cells, or hemocytoblasts, according to Maximow.<sup>16</sup> By others—especially the dualists—they have been called "micromyeloblasts." These cells are smaller than lymphocytes and have a dense nucleus composed of dense chromatin masses. Although practically devoid of cytoplasm a thin blue rim is occasionally seen. In supravital preparations they appear as small nuclear masses with little distinct cytoplasm in which neither mitochondria, granules, nor vacuoles are found. Their true significance is unknown. Nordenson's term, "hematogonia," has been adopted for use in this paper. No transitions from these cells to myeloid cells could be seen in the present series of cases.

5. *Reticulum Cells*. These cells have a relatively large amount of cytoplasm, which, in the present series, was granular and dirty grayish-blue in color. The cytoplasmic margins were often serrated. The nucleus was ovoid in shape with the chromatin material loosely packed in clumps. Ingested debris was frequently found in the cytoplasm.

*Endothelial Cells*. The endothelial cells were elongated, with an elliptical nucleus and with the cytoplasm tapering to pointed ends. The nucleus had large chromatin clumps and the cytoplasm was finely granular and light grayish in color.

6. *Megakaryocytes*. These are the largest of the marrow cells, having finely granular basophilic cytoplasm, with either small separate or connected violet-colored homogeneous nuclei. Some of the megakaryocytes had a deeper and clearer blue-colored cytoplasm with huge dark violet-colored nuclei. These may be regarded as megakaryoblasts.

7. *Gaucher Cells*. These atypical cells were large cells with a bluish white cytoplasm of a fibrillar structure. Some of these cells may contain small vacuoles with small nuclei often pushed eccentrically by the fibrillar substance.

8. *Normal and Nucleated Red Blood Cells.* There is a difference of opinion in respect to nomenclature for the development stages of the red cells. The most primitive types are the largest and are devoid of hemoglobin. They are found in large numbers only in pernicious anemia and sprue. Mitoses are common and indicate either transition or maturation to smaller cells. In the latter cells there is a slow deposition of hemoglobin.

The following classification is based on the character of the nucleus rather than the size of the red cells:

A. *Megaloblasts.* These cells were large. The cytoplasm was of a deep blue color without vacuoles or granules. The nucleus was large and had a characteristic loose, finely meshed chromatin network, somewhat resembling a sieve. Nucleoli, when present, were very large. These cells, at times, were difficult to distinguish from the myeloblasts.

B. *Erythroblasts.* These appeared similar, but smaller than megaloblasts. The nucleus had a denser or more compact chromatin network, but still retained the sieve-like appearance. Occasionally, hemoglobin formation could be seen developing in a portion of the blue cytoplasm.

C. *Normoblasts.* These were more or less hemoglobiniferous cells containing a pyknotic nucleus. They varied considerably in size.

#### CLINICAL MATERIAL

Our observations are based on the study of 246 cases. Sternal puncture was done on 21 normal persons, who served as controls; the others were pathological cases, some of whom had repeated sternal punctures. The cases, with respective clinical diagnoses are listed in Table 1.

Summaries of the clinical and laboratory findings of representative cases in the above-listed groups follow:

#### I. NORMAL CASES

Twenty-one cases—thirteen females and eight males. Most of these were laboratory and hospital employees. Four were donors who had not given blood for transfusions for at least two months preceding the sternal puncture. Two of the authors were also included in this group, and from actual experience, the procedure was found to be almost painless, even without anesthesia, though there is a peculiar feeling of discomfort when the marrow fluid is withdrawn.

It will be noted that our percentages of nucleated red cells, in proportion to the white cells, compare favorably with those of Arinkin<sup>2</sup> and Nordenson.<sup>17</sup> The percentage of various white cells may show some differences as a result of our cellular classification. For example, we have included the metamyelocytes among the myelocytes. The non-segmented form percentages are higher than

TABLE 1

<b>I. NORMAL:</b>	
Men.....	8
Women.....	13
<b>II. PATHOLOGICAL:</b>	
<b>A. White Cell Disturbances:</b>	
1. Leukemia:	
(a) Myeloid leukopenic.....	8
Myeloid leukocythemie.....	13
(b) Lymphatic leukopenic.....	1
Lymphatic leukocythemie.....	5
Lymphatic acute.....	1
(c) Monocytic.....	1
(d) Leukemia with remission.....	1
2. Infectious mononucleosis.....	6
3. Post-infectious lymphocytosis.....	1
4. Eosinophilia (peripheral).....	7
5. Monocytosis (peripheral).....	5
6. Agranulocytosis.....	3
7. Secondary agranulocytosis (severe leukopenia).....	2
8. Monocytic agranulocytosis (leukopenic infectious monocytosis).....	1
<b>B. Red Cell Disturbances:</b>	
1. Pernicious anemia:	
Treated.....	10
Untreated.....	7
2. Sprue:	
Treated.....	3
Untreated.....	2
3. Hemolytic jaundice:	
Splenectomized.....	1
Non-splenectomized.....	8
4. Hemolytic anemia.....	4
5. Sickle-cell anemia.....	2
6. Polycythemia:	
Treated.....	12
Untreated.....	3
Spent.....	2
Secondary.....	2
7. Secondary anemia (achlorhydric-nutritional, etc.).....	12
8. Chronic congenital aregenerative anemia.....	1
9. Aplastic anemia.....	3
<b>C. Platelet Disturbances:</b>	
1. Thrombocytopenic purpura hemorrhagica:	
(a) Before and after splenectomy.....	3
(b) Chronic.....	4
(c) Secondary.....	2

TABLE 1—*Concluded*II. PATHOLOGICAL—*Concluded*:D. *Splenopathies*:

1. Gaucher's . . . . .	4
2. Hodgkin's (3 treated with x-ray) . . . . .	5
3. Splenomegaly (splenic vein thrombosis—4) . . . . .	15
4. Malaria . . . . .	2
5. Schistosomiasis . . . . .	1
6. Undulant fever . . . . .	1

E. *Neoplasms*:

1. Myeloma . . . . .	4
2. Carcinoma . . . . .	12
3. Sarcoma (lymphosarcoma—4; and follicular lymphoblastoma—2) . . . . .	8
4. Fibro-myoma of stomach . . . . .	1

F. *Lues* . . . . .

4

G. *Miscellaneous Diseases*:

1. Diseases with "shift to right" . . . . .	5
2. Diseases with "shift to left" . . . . .	6
3. Diseases with no shift . . . . .	17

H. *Specific Disease Entities*:

1. Leprosy . . . . .	1
2. Prematurity . . . . .	2
3. Filariasis . . . . .	1

Total . . . . . 246

Nordenson's<sup>17</sup> rod forms, since he places some of these cells in the metamyelocyte group. Approximately 90 per cent of the cells in normal bone marrow consist of myeloid cells distributed as follows: myelocytes (22 per cent), non-segmented neutrophils (29.9 per cent), and segmented neutrophils (32.1 per cent). The myeloblasts and promyelocytes, eosinophilic myelocytes and basophilic myelocytes, and mature eosinophils and basophils, comprise about 1 per cent respectively, with the lymphocytes (7 per cent), hematogones (less than 2 per cent), and megakaryocytes (0.2 per cent) making up the remaining 10 per cent.

The nucleated red cells are normally present in the ratio of 1 nucleated red cell to 3 white cells. Approximately 75 per cent of the nucleated red cells are normoblasts, 25 per cent erythroblasts and 0.5 per cent megaloblasts.

## II. PATHOLOGICAL CASES

A. *White Blood Cell Disturbances*

1. *Leukemia*. (a) *Myeloid Leukemia*. Twenty-one cases were studied. Eight had leukopenic peripheral counts, and in thirteen, the count was leukocythemic. In the acute myeloid leukemic cases the peripheral white cell counts varied between 90,000 and 200,000; the chronic cases, between 20,000 and 60,000. The leukopenic cases varied between 700 and 8,000. In the

acute cases, the marrow findings disclosed a differential count with myeloblasts varying between 88 and 99 per cent (table 4). The chronic cases revealed a high myelocytic differential. The acute and chronic leukopenic myeloid leukemia cases had marrow differentials not unlike the acute and chronic leuko-

TABLE 2  
NORMALS

	ARINKIN	NORDENSON	AUTHORS	AUTHORS (AVERAGE)
Myeloblasts.....	1.0- 2.4	0.25- 5.5	0.4- 5.0	1.6
Promyelocytes neutrophilic.....	1.0- 2.8	1.25- 8.25		
Myelocytes agranulocytic.....			0 - 4.0	0.1
Myelocytes:				
Neutrophilic.....	4.5- 8.6	4.25- 18.0	12.0-32.0	21.5
Eosinophilic.....	0.3- 1.0	0 - 6.25	2.0- 2.2	0.77
Basophilic.....		0 - 0.5		
Metamyelocytes:				
Neutrophilic.....	1.4- 3.4	12.5 - 42.5		
Eosinophilic.....	0.3- 1.0			
Basophilic.....				
Non-segmented:				
Neutrophiles.....		2.25- 10.75	18.0-40.0	30.2
Eosinophiles.....			0 - 2.2	0.39
Segmented:				
Neutrophiles.....	41.5-55.0	14.25- 35.0	20.0-50.0	34.0
Eosinophiles.....	0.6- 4.0	0.25- 7.5	0.2- 2.8	0.94
Basophiles.....	0 - 0.7	0 - 0.75	0 - 1.0	0.07
Lymphocytes.....	7.3-16.5	7.5 - 38.0	1.0-19.0	8.6
Monocytes.....	2.1- 9.3	0 - 5.0		
Plasma cells.....	0.3- 0.9	0 - 3.25		
Reticulum cells.....			0 - 1.2	0.25
Unclassified (hematogones).....		0 - 4.5	0 -10.6	3.1
Megakaryocytes.....	0.6- 6.1	0 - 1.0	0 - 0.8	0.2
Ferrata cells.....		3.0 - 40.0		
Megaloblasts.....			0 - 1.4	0.14
Pronormoblasts.....	0.8- 2.9	0 - 6.0		
Normoblasts basophilic.....		1.0 - 16.0		
Erythroblasts.....			1.2-23.0	7.1
Normoblasts.....	5.7-16.0	26.0 -184.0	7.0-60.0	22.6

Average marrow white blood cells—118,750 per cubic millimeter.

cythemic leukemias. In general, erythropoiesis was diminished and few megakaryocytes were seen. In some of the chronic cases, there was an increase in basophils and basophilic myelocytes, in one case as high as 20 per cent. This coincided with the peripheral blood count. In leukemic patients

treated with x-ray therapy the bone marrow findings were similar to the untreated cases, even when the peripheral white blood cell count had been reduced.

In all but two cases, the diagnosis of leukemia had been established from the peripheral count, and the marrow findings gave only confirmatory evidence. In two cases, the diagnosis, previous to marrow puncture, was purpura hemorrhagica and agranulocytosis:

*Case E. G. (Method 2). Acute Leukocythemic Myeloblastic Leukemia.* This previously healthy, well-developed, precocious 14 year old girl developed acute generalized lymphadenopathy and infectious mononucleosis was suspected before the peripheral count was made. The patient died three weeks after the onset of initial symptoms. The sternal marrow puncture revealed 99 per cent myeloblasts.

*Case B. G. (Method 1). Acute Leukopenic Myeloid Leukemia.* This pale, poorly-nourished 41 year old woman, had a history of menorrhagia and purpuric spots for four years and a recent history of weakness and anemia. There was no splenomegaly or lymphadenopathy. The history and clinical findings suggested the diagnosis of chronic purpura hemorrhagica. The peripheral blood picture suggested leukopenic myeloid leukemia but the sternal aspiration immediately gave the correct diagnosis. The differential marrow count showed 90 per cent myeloblasts.

*(b) Lymphatic Leukemia.* Seven cases were studied, one subleukemic and five chronic leukocythemic and one lymphoblastic. The expected increase in lymphocytes was present. Many of the lymphocytes had bi-lobed nuclei, and many had nucleoli. It was difficult to ascertain whether these cells were lymphoblasts, and therefore they were not so classified. The case of acute lymphoblastic leukemia had 60 per cent of definite lymphoblasts, with marked increase in mitotic figures. Erythropoiesis was decreased.

*Case L. L. (Method 2).* A well-developed, well-nourished, 38 year old man complained of recent weakness and fatigue. The lymph nodes and spleen were palpable. The marrow differential revealed over 80 per cent lymphocytes.

*(c) Monocytic Leukemia. Case A. V.* A 37 year old, well-developed colored female presented typical signs of monocytic leukemia, such as buccal mucosal ulcerations, enlargement of lymph nodes, anemia, fever and splenomegaly. The marrow findings in this case were very helpful in establishing the diagnosis of monocytic leukemia: Monocytic myeloblasts 45 per cent; monocytes 22 per cent.

*(d) Leukemia with Remission (Monocytoid Myeloblastic Leukemia). Case I. W.* A 44 year old, well-nourished nurse was an unusually interesting case for this study. She entered the hospital in July, 1935, with a history of increasing weakness, dyspnea and pallor for the preceding six months. During this period the patient noted non-inflammatory skin elevations over the arms and trunk. The physical examination revealed pallor, slight enlargement of the peripheral nodes and yellowish infiltrating skin nodules over the trunk and



upper extremities. A white count at this time revealed 5,000 white cells with 40 per cent monocytoïd myeloblasts. This atypical cell was found infiltrating the skin, (biopsy), lymph nodes (biopsy) and bone marrow (puncture). The patient then developed a broncho-pneumonia at which time the count dropped to 500 white cells per cubic millimeter, with a relative lymphocytosis. Following many transfusions the patient improved, with a disappearance of the atypical cells in the skin, peripheral blood and marrow. During the following nine months the patient appeared normal and continued with her routine work and the blood and marrow findings on two occasions were normal. Again, in April 1936 (ten months after the initial symptoms) the patient developed a recurrence of her original condition, with skin infiltrations, and involvement of the blood and marrow. The case is now being carefully followed. Similar cases have been reported by Jackson.<sup>13</sup>

2. *Infectious Mononucleosis*. Six cases were studied. The marrow findings showed an increase in lymphocytes. Many of the lymphocytes were of the lymphoid type. One case had 7 per cent Turk irritation cells in the peripheral blood, but none were found in the marrow. Another had 23 per cent Turk irritation cells in the peripheral blood and 27 per cent in the marrow.

3. *Post-infectious Lymphocytosis*. This case had an exaggerated lymphatic response following a severe infection with an increase in lymphocytes of the marrow.

4. *Eosinophilia*. Seven cases were studied. (Two cases of dermatitis exfoliativa, three unexplained eosinophilia, one case of trichinosis, and one case of periarteritis nodosa.) These patients had between 17 per cent and 60 per cent eosinophils in the peripheral blood. A correlation between the peripheral and marrow eosinophilia was not always present.

*Case P. D.* (Method 2). A 32 year old policeman with severe chronic asthma, of three years duration, entered the hospital for asthma, weakness and ascites. A muscle biopsy confirmed the clinical impression of periarteritis nodosa.

5. *Monocytosis*. Five cases were studied: two, of subacute bacterial endocarditis; one, of ileocolitis; one, unexplained monocytosis; and one case that had radium treatment for nasal hemorrhages. The monocytes in the peripheral blood ranged from 18 per cent to 36 per cent. In none of these cases did the marrow show an increase in monocytes (Doan and Wiseman<sup>6</sup>).

*Case R. H.* (Method 1). A pale, emaciated boy 14 years of age, developed an ulcerative colitis three years before admission, and his condition had become gradually worse, requiring transfusions and frequent hospitalization. The peripheral count revealed 24 per cent monocytes but no monocytes were found in the aspirated marrow.

6. *Agranulocytosis*. A study of three cases was made. One followed neosphenamine treatment, one gave a history of taking dinitrophenol, and the other had taken large amounts of pyramidon. These cases revealed few granulocytes in the marrow. Lymphocytes predominated.

*Case R. B.* (Method 2). A well-developed Spanish cook, aged 42 began his

first antileptic treatment six weeks before entering the hospital. He was admitted because of marked dyspnea, fever and prostration. Death occurred two days later. Autopsy confirmed the diagnosis.

*Peripheral Blood.* Hemoglobin 82 per cent; red blood cells 4,310,000; white blood cells 450; platelets 210,000. *Differential:* Non-segmented neutrophils 2 per cent; segmented neutrophils 6 per cent; eosinophils 1 per cent; lymphocytes 91 per cent.

*Marrow.* Hemoglobin 80 per cent; red blood cells 5,000,000; total nucleated cells 1,600; platelets 140,000; red cell volume 31 per cent; white cell volume 1 per cent; total white blood cells 1,000. *Differential:* Myeloblasts 5 per cent; neutrophilic myelocytes 6 per cent; non-segmented neutrophils 3 per cent; segmented neutrophils 1 per cent; segmented eosinophils 0.6 per cent; lymphocytes 80 per cent; plasma 1 per cent; monocytes 0.3 per cent; hematogones 3 per cent; reticulum cells 1 per cent; megakaryocytes 0.2 per cent; megaloblasts 0.6 per cent; total nucleated red cells 600; erythroblasts 43 per 100 white cells; normoblasts 6 per 100 white cells.

7. *Secondary Agranulocytosis (Severe Leukopenia).* Two cases presented marked leukopenia (800 white blood cells) and neutropenia (20 per cent polymorphonuclears). In both the marrow was hyperplastic with a relatively normal number of myeloid elements. One of these patients improved and the other died. Permission was not obtained for an autopsy but, clinically, the case was diagnosed as septicemia since blood cultures were the only positive findings. The following is the case history of the patient who improved:

*Case D. A. (Method 1).* A well-developed, apparently healthy woman, 44 years of age, had a negative history until two months prior to admission. At that time she developed influenza, following which she has been almost helplessly weak. Her spleen was palpable. Five months after puncture, patient improved, clinically and hematologically. The peripheral white count was 2,100.

*Peripheral Blood.* Hemoglobin 73 per cent; red blood cells 5,650,000; white blood cells 800; platelets 90,000. *Differential:* Toxic non-segmented neutrophils 9 per cent; toxic segmented neutrophils 11 per cent; lymphocytes 56 per cent; large lymphocytes 12 per cent; plasma 1 per cent; monocytes 10 per cent.

*Marrow.* Hemoglobin 68 per cent; red blood cells 3,320,000; total nucleated cells 34,000; platelets 150,000; red cell volume 25 per cent; white cell volume 3 per cent; total white cells 27,400. *Differential:* Neutrophilic myelocytes 33 per cent; non-segmented neutrophils 54 per cent; segmented neutrophils 4 per cent; eosinophilic myelocytes 1.6 per cent; non-segmented eosinophils 2.4 per cent; segmented eosinophils 2.8 per cent; lymphocytes 4.4 per cent; total nucleated red cells 6,600; erythroblasts 7 per 100 white cells; normoblasts 17 per 100 white cells.

8. *Monocytic Agranulocytosis (Leukopenic Infectious Monocytosis).* *Case M. G. (Method 1).* A well-nourished woman, 42 years of age, received a painful injury to her shoulder two months prior to admission. The pain was controlled

by pyramidon (two dozen 5 grain tablets). Eighteen days before entering the hospital, she developed sore throat, swollen painful cervical glands, and sore gums. Otherwise, the physical examination was negative. Pyramidon was again prescribed and the condition became worse, the tonsils and gums becoming ulcerated and necrotic. After entering the hospital, the blood count revealed a leukopenia with a relative and absolute increase in monocytes. This was considered a benign type of agranulocytosis (Rosenthal<sup>23</sup>) and (Rosenthal and Abel<sup>24</sup>). We have not had the opportunity to test the sensitivity of this patient to pyramidon, but we believe that no relationship exists. The peripheral count revealed 54 per cent monocytes while the marrow showed 2.6 per cent monocytes which may have been due to admixture of blood. Following the second marrow puncture, 10 days later, no monocytes were found.

*(To be continued)*

## NEWS AND NOTICES

At the Annual Banquet given during the convention of the American Society of Medical Technologists in Atlantic City, the principal speakers were Dr. Frank W. Konzelmann of Temple University School of Medicine, Philadelphia, and Dr. Robert A. Kilduffe, of Atlantic City.

Dr. Konzelmann spoke on "Organization," Dr. Kilduffe upon "The Function of The Clinical Laboratory."

Among those present were Drs. Hillkowitz, Kracke, Maynard, Moore, Yaguda, and Boerner.

The Convention was well attended and featured by exhibits of outstanding merit.

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A Section on Clinical Pathology of The Philadelphia County Medical Society was recently organized in Philadelphia with the following officers: Chairman, Dr. Kenneth Fowler; Vice-Chairman, Dr. John D. Paul; Secretary, Dr. William L. C. Spaeth.

The following representatives to the Commission on Medical Economics of The Philadelphia County Medical Society were elected:

Dr. Herbert Fox, Honorary Chairman  
Dr. Frank W. Konzelmann, Chairman  
Dr. Charles W. Lueders  
Dr. Jefferson H. Clark  
Dr. Max Strumia  
Dr. John Eiman

### REPORTS ON ANTI-POLIOMYELITIS PROPHYLACTIC SPRAY

Current studies reveal there have been fewer infantile paralysis cases among those sprayed with the newly developed picric acid-alum solution than among those who were not sprayed in the previous Alabama experiments. The results were such as to cause the recommendation to be made by Dr. Charles Armstrong of the United States Public Health Service that investigation be continued to find the most effective method of application and the ideal solution. Dr. Armstrong is co-developer of the spray and actively in charge of the Alabama experiments. His recommendation is contained in the Transactions of Southern Branch, American Public Health Association, recently made public.

The experiments constituted the first large scale trial of this solution on humans. Drs. Armstrong and W. T. Harrison also of the U. S. Public Health Service, first had carried on experiments with monkeys, saving 24 out

of 25 sprayed animals infected with the poliomyelitis, or infantile paralysis virus, while 20 of 26 infected and not previously sprayed, died. No demonstrable local effects were noted on the sprayed monkeys.

When the Alabama outbreak of infantile paralysis came it was decided to carry out field trials, but, Dr. Armstrong says in his report: "It was made clear that the evidence as to protective action of the proposed spray was based entirely upon animal experimentation and was not to be considered of proven value in the prevention of poliomyelitis in man."

When the seriousness of the outbreak in Alabama became apparent, all of the 30,000-odd W.P.A. workers and their families were sprayed every other day at first, and then once a week. In addition 91 W.P.A. nurses were detailed to counties without health units, not only to spray the W.P.A. workers and their families, but to help care for victims of the disease.

Dr. Armstrong kept careful laboratory check of the results in Birmingham and the surrounding territory of Jefferson County.

Of a total of 5,097 white persons, 3,545 or 61.9 per cent, were sprayed some time between July 15 and August 22, while of 2,996 colored persons, 1,465, or 48.9 per cent had been sprayed.

Physicians in Alabama, as well as Tennessee and Mississippi where there also were infantile paralysis outbreaks, particularly were requested to report any cases of untoward results of the use of the spray. They reported only seven such cases among an estimated 2,000,000 persons who were sprayed in the three states. These seven cases of untoward results were not of a serious nature.

In making his compilations, Dr. Armstrong considered every person, however inadequately sprayed, to be in the sprayed class. Also, every case that developed after spraying, however faulty or inadequate the method, was attributed to the sprayed group, although it was assumed that it took two weeks for the spray to become effective and cases developing within that time were excluded from the sprayed group.

On this basis, seven cases were found within the sprayed group, while theoretically 11.4 cases might have been expected, these data being based on weeks of life and the number of cases in the non-sprayed group. All of the seven had been home-sprayed.

After a general discussion of the problems involved in the experiments, Dr. Armstrong makes the following finding and recommendations:

"1. Chemicals capable of blocking the olfactory route of infection must be thoroughly applied to the nasal vault if maximum protection is to be secured.

"2. Many children actively resist and thus render spraying difficult.

"3. Sympathetic parents, unfamiliar with the anatomy of the nose, are not, as a class, qualified to administer intranasal prophylactics properly.

"4. A house-to-house survey revealed complaints by 885 from among 4,631 sprayed individuals. Headache, temporary nausea, burning of nostrils, symptoms of head cold, irritated throat, and irritation of eyes, in the order named,

were the most unusual complaints. Had the applications of the chemicals been more uniformly thorough more unpleasant consequences might have developed.

"5. Seven instances of hypersensitivity or of idiosyncrasy to the drugs were reported from the whole epidemic area.

"6. The actual incidence of poliomyelitis in the group sprayed by whatever method was somewhat less than the calculated incidence based upon the rate in the unsprayed control group (16.21.7) (Birmingham area).

"7. The occurrence of cases in persons who had sprayed for several weeks in the advised manner throws question upon the method as employed.

"8. In the face of an epidemic of poliomyelitis the people can be relied upon to employ any simple, inexpensive prophylactic method of promise.

"9. It seems probable that the most effective method of application, as well as the most ideal solution, has not yet been found. Investigation should therefore be continued."

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At the recent A. M. A. Convention in Atlantic City the Silver Medal in Class Two was awarded to F. W. Hartman, M.D., of the Henry Ford Hospital, Detroit, for an exhibit showing a new low cost oxygen tent using liquid air and liquid oxygen.

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Dr. Thomas B. Magath of the Mayo Clinic has been appointed Health Officer of the city of Rochester. He succeeds Dr. Charles H. Mayo who has been Health Officer for twenty-five years.

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Emmerich von Haam, M.D., formerly of Tulane University, has been appointed to the Professorship in Pathology at Ohio State University, assuming his new duties as of July 1st, 1937.

#### WELCOME!

The following new members were elected at the recent Convention in Philadelphia:

##### *Honorary Membership:*

Dr. Cuthbert Dukes.....	London, England
Dr. Niels P. Dungal.....	Reykjavik, Iceland
Dr. S. C. Dyke.....	Wolverhampton, England
Dr. Joseph McFarland.....	Philadelphia, Pennsylvania
Dr. Charles Sheard.....	Rochester, Minnesota

##### *Associate Membership:*

Dr. Harold A. Abel.....	New York, New York
Dr. Charles A. Doan.....	Columbus, Ohio



Dr. T. J. Domanski.....	Jersey City, New Jersey
Dr. William A. Groat.....	Syracuse, New York
Dr. Joseph F. Hamilton.....	Memphis, Tennessee
Dr. Charles W. Lueders.....	Philadelphia, Pennsylvania
Dr. Salem M. Rabson.....	New York, New York
Dr. E. A. Sharp.....	Detroit, Michigan
Dr. Charles H. Watkins.....	Rochester, Minnesota

*Regular Membership:*

Dr. Wm. Arnold Antopol.....	Newark, New Jersey
Dr. Carl W. Apfelbach.....	Chicago, Illinois
Dr. Aaron Arkin.....	Chicago, Illinois
Dr. William Aronson.....	New York, New York
Dr. Stanley M. Asselstine.....	Windsor, Ontario, Canada
Dr. John T. Bauer.....	Philadelphia, Pa.
Dr. James S. P. Beck.....	Worcester, Mass.
Dr. William G. Bernhard.....	Newark, New Jersey
Dr. Leon L. Blum.....	Terre Haute, Indiana
Dr. George C. Bower.....	Marcy, New York
Dr. Robert N. Brown.....	Dayton, Ohio
Dr. S. W. Budd.....	Richmond, Virginia
Dr. Harry L. Clark.....	Detroit, Michigan
Dr. Mortimer Cohen.....	Pittsburgh, Pa.
Dr. Richard W. Cragg.....	Rochester, Minnesota
Dr. O. I. Cutler.....	Loma Linda, Cal.
Dr. Elbert De Coursey.....	Washington, D. C.
Dr. P. Arthur Delaney.....	Chicago, Illinois
Dr. George H. Fetterman.....	Mayview, Pa.
Dr. Henry S. Glidden.....	Tewksbury, Mass.
Dr. Ferdinand C. Helwig.....	Kansas City, Mo.
Dr. John C. Henthorne.....	Rochester, Minnesota
Dr. Wayne M. Hull.....	Oklahoma City, Oklahoma
Dr. Jesse H. Inman.....	Bakersfield, Cal.
Dr. Herman B. Kaplan.....	Union City, New Jersey
Dr. Donald H. Kaump.....	Rochester, Minn.
Dr. Louisa E. Keasbey.....	Lancaster, Pa.
Dr. John A. Lanford.....	New Orleans, La.
Dr. Aaron Learner.....	Chicago, Illinois
Dr. H. H. Leffler.....	Washington, D. C.
Dr. Nathaniel H. Lufkin.....	Minneapolis, Minn.
Dr. Herbert Lund.....	Uniontown, Pa.
Dr. F. W. Luney.....	London, Canada
Dr. W. L. Marr.....	Galveston, Texas

Dr. Thomas W. McCreary.....	Monaca, Pa.
Dr. John R. McDonald.....	Rochester, Minn.
Dr. John King Miller.....	Louisville, Ky.
Dr. Laurence C. Milstead.....	Allentown, Pa.
Dr. Harold W. Morgan.....	Mason City, Iowa
Dr. Arch H. Morrell.....	Augusta, Me.
Dr. Ralph Mosteller.....	Spartanburg, S. C.
Dr. V. R. Naidu.....	Rochester, Minn.
Dr. Robert J. Needles.....	Detroit, Michigan
Dr. Sophie T. Nowakovsky.....	Chicago, Illinois
Dr. Michael A. Ogden.....	New Orleans, Louisiana
Dr. Orin A. Ogilvie.....	Salt Lake City, Utah
Dr. Rudolf M. Paltauf.....	New York, New York
Dr. Thomas K. Rathmell.....	Norristown, Pa.
Dr. James Y. Rodger.....	Lowell, Mass.
Dr. Herbert J. Schattenberg.....	New Orleans, La.
Dr. Paul G. F. Schmitt.....	Chicago, Illinois
Dr. Etta B. Selsam.....	Terre Haute, Indiana
Dr. Will Shimer.....	Indianapolis, Indiana
Dr. Marshall W. Sinclair.....	Bluefield, West Virginia
Dr. Charles W. Stewart.....	Baltimore, Maryland
Dr. Max M. Strumia.....	Bryn Mawr, Pa.
Dr. Jacob Taub.....	Bronx, New York
Dr. Frances DeBone Taylor.....	Philipsburg, Pa.
Dr. Wilbur C. Thatcher.....	Iowa City, Iowa
Dr. Leander J. Van Hecke.....	Wauwatosa, Wis.

#### THE LABORATORY AND THE SOCIAL SECURITY ACT

Operators of private laboratories, private sanitariums, and physicians employing one or more persons are advised by the Commissioner of Internal Revenue to make immediate tax returns as required under the provisions of Titles VIII and IX of the Social Security Act to avoid further payment of drastic penalties which are now accruing.

The Commissioner pointed out that every person employed in such work came under the provisions of Title VIII, which imposes an income tax on the wages of every taxable individual and an excise tax on the pay roll of every employer of one or more. This tax is payable monthly at the office of the Collector of Internal Revenue. The present rate for employer and employee alike is one per cent of the taxable wages paid and received.

Under Title IX of the Act, employers of eight or more persons must pay an excise tax on their annual pay roll. This tax went into effect on January 1, 1936, and tax payments were due from the employers, and the employers alone, at the office of the Collector of Internal Revenue on the first of this year.

This tax is payable annually, although the employer may elect to pay it in regular quarterly installments.

The employer is held responsible for the collection of his employee's tax under Title VIII and is required to collect it when the wages are paid the employee, whether it be weekly or semi-monthly. Once the employer makes the one per cent deduction from the employee's pay, he becomes the custodian of Federal Funds and must account for them to the Bureau of Internal Revenue.

This is done when the employer makes out Treasury form SS-1, which, accompanied by the employee-employer tax, is filed during the month directly following the month in which the taxes were collected. All tax payments must be made at the office of the Collector of Internal Revenue in the district in which the employer's place of business is located.

*Penalties for delinquencies are levied against the employer, not the employee and range from 5 per cent to 25 per cent of the tax due, depending on the period of delinquency. Criminal action may be taken against those who willfully refuse to pay their taxes.*

The employers of one or more are also required to file Treasury forms SS-2 and SS-2a. Both are informational forms and must be filed at Collectors' offices not later than July 31, covering the first six months of the year.

After that they are to be filed at regular quarterly intervals. Form SS-2 will show all the taxable wages paid to all employees and SS-2a the taxable wages paid each employee.

Participation in a state unemployment compensation fund, approved by the Social Security Board, does not exempt employers from the excise tax under Title IX. Nor does the fact that there is no state unemployment compensation fund relieve the employer of his Federal tax payments. In those states where an unemployment compensation fund has been approved, deductions up to 90 per cent of the Federal tax are allowed the employer who has already paid his state tax. *These deductions are not allowed unless the state tax has been paid.*

This tax is due in full from all employers in states having no approved fund. The rate for 1936 was one per cent of the total annual pay roll containing eight or more employees, and for 1937 it is two per cent. The rate increases to three per cent in 1938 when it reaches its maximum. The annual returns are made on Treasury form 940.

An employer who employs eight or more persons on each of twenty calendar days during a calendar year, each day being in a different calendar week, is liable to the tax. The same persons do not have to be employed during that period, nor do the hours of employment have to be the same.

Actual money, when paid as wages, is not the sole basis on which the tax is levied. Goods, clothing, lodging, if a part of compensation for services, are wages and a fair and reasonable value must be arrived at and become subject to the tax.

Commissions on sales, bonuses and premiums on insurance are wages and taxable.

Officers of corporations whether or not receiving compensation are considered employees for the purpose of taxation.

Wages paid during sick leave or vacation, or at dismissal are taxable.

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Arrangements are being made to hold the fourth International Leprosy Conference in Cairo, beginning the 21st of March 1938. This conference is being organized by the International Leprosy Association, and this will be the first International Conference to be arranged by this association since its inauguration in 1931. Three previous conferences of this nature have been held—at Berlin in 1897, at Bergen in 1909 and at Strassbourg in 1923.

¶ The Egyptian Government is inviting all countries concerned to send official delegates. In addition to these, doctors and others interested in the subject are invited to be present. Full information can be obtained from the Secretary of the International Leprosy Association, 131 Baker Street, London, W. 1.

## BOOK REVIEWS

*The Toxemias of Pregnancy.* By DAME LOUISE McIRAY, Consulting Obstetrician and Gynecological Surgeon, Royal Free Hospital, etc. Cloth, 355 pp., 18 figures, \$5.00. William Wood & Co., Baltimore.

This book is based upon lectures upon the toxemias of pregnancy given by the author in the University of Birmingham to which considerable material has been added. The book is not intended as a text for students but rather as a reference text for obstetricians, physicians, and biochemists confronted with the problems involved in the complications of pregnancy.

The author writes from an extensive experience and has produced a text of definite value which well repays reading.

*The Cardiac Glucosides.* By PROFESSOR ARTHUR STOLL. The Pharmaceutical Press, London.

This volume contains a series of three lectures delivered in the College of The Pharmaceutical Society of Great Britain under the auspices of The University of London. It discusses previous investigations of the cardiac glucosides, including those of the author.

*Electrocardiography.* By C. C. MAHER, Assistant Professor of Medicine, Northwestern University. Cloth, Ed. 2, 254 pp., 95 illustrations, \$4.00. William Wood & Co., Baltimore.

It was inevitable that this book should reach a second edition as it is one of the most practical and clearly written expositions of a complicated subject that this reviewer has had opportunity to see.

To the physician, student, and all who are interested in electrocardiography it may be recommended as a safe and authoritative guide.

*Physicians and Medical Care.* By ESTER LUCILLE BROWN, Department of Statistics, Russell Sage Foundation. Cloth, 202 pp., 75 cents. Russell Sage Foundation, New York.

This is one of a series of monographs summarizing the results of studies by the Russell Sage Foundation.

To those interested in medical economics—and who is not?—this little book will be of interest.

*Aids to Pathology.* By HARRY CAMPBELL, M.D., F.R.C.P., and KENNETH CAMPBELL, F.R.C.S. Cloth, Ed. 7, pp. 263, 12 figures, \$1.50. Baillière, Tindall & Cox, London; Williams & Wilkins Co., Baltimore.

That this small handbook, intended to serve as a "refresher" for student and practitioner, has well served its purpose is evidenced by the fact that it has reached a seventh edition.

*The Biochemistry of Medicine.* By A. T. CAMERON, Professor of Biochemistry, University of Manitoba, and C. R. GILMOUR, Professor of Medicine, University of Manitoba. Ed. 2, Cloth, 518 pp., 31 illustrations, \$6.00. William Wood & Co., Baltimore.

This book needs no introduction and this second edition, revised and rewritten to embody the new advances, like the first edition, without doubt will take its place as a standard reference text.

Well written, comprehensive, and authoritative, the physician should welcome this useful text for its practical viewpoint and for its practical application to the everyday practice of medicine.

*Physiological Chemistry.* By J. F. McLENDON, Professor of Physiological Chemistry, Medical School, University of Minnesota, and the late C. J. V. PETTIBONE. Cloth, Ed. 6, 454 pp., 33 figures, \$3.50. The C. V. Mosby Co., St. Louis, Mo.

The Sixth Edition of this well known text, extensively revised and largely rewritten, may be recommended as a clear, comprehensive, and authoritative text book. While primarily intended as a student manual, it will prove of value to the laboratory worker as well and may be expected to achieve the enviable repute and standing of previous editions.

*Death Rides with Venus.* By ARTHUR C. PALM, Director of The Health Foundation, Cleveland. Cloth, 157 pp., \$1.50. The Greystone Press, New York.

It is, perhaps, inevitable that the present campaign against syphilis will produce an avalanche of books of this type as it has already produced a flood of newspaper and magazine articles.

It is also perhaps inevitable that many of them—like this one—will be patterned more or less after the now familiar de Kruif formula: One takes a grain or two of truth—it doesn't have to be much—and with this as a foundation, erects upon it a tremendous structure, which is then viewed with alarm heavily tintured with horror.

It is necessary for the author of this type of writing to accompany his viewing with anguished groans, frantic shrieks of despair, and scathing denunciation in the style of the pulp magazines of the confession and muck-raking type.

Without de Kruif's facility of anguish and invective—which, by last reports seems to be growing somewhat threadbare—Mr. Palm nevertheless achieves a fairly successful though, by comparison, somewhat pallid effect. According to Mr. Palm there is nothing mysterious or complicated about the prevalence of gonorrhea and syphilis. They may be laid at the door of the doctor and



attributed to medical ignorance, excessive fees, lack of free clinics, and poor training in the medical schools.

As some one once said of something or other: "It may be magnificent—but is it art?" We might say to Mr. Palm: "This is indeed terrible—but is it, as a matter of fact, entirely true?"

In justice to the author it must be added that he is sincere in his belief and frank in his expression of it. He, at least, is not guilty of intellectual dishonesty.

## EDITORIAL

### THE SYPHILIS CAMPAIGN AND THE CLINICAL PATHOLOGIST

Under the able leadership of Surgeon General Thomas Parran, of the United States Public Health Service, plans are being formulated for a campaign for the control and eradication of syphilis in the United States.

Neither the importance nor the magnitude of the struggle to be initiated against this disease, require emphasis or elaboration, certainly not to the physician, and especially not to the clinical pathologist.

The problem is so immense, however, and presents so many difficulties—some obvious and others apparent only upon consideration of its many inevitable ramifications—that the essential necessity for the entire coöperation of both the public and the medical profession is at once apparent. It is incumbent upon the profession at large, and, it may be added, upon the clinical pathologist in particular, to visualize the problem not only as a whole but also as its various and particular phases apply to the individual; only by such consideration can coöperation be made effective.

In its broadest aspects the success or failure of a campaign against syphilis must depend upon: (a) the consistent and persistent education of the public as to the nature of the disease; (b) the full utilization of the various procedures applicable to its recognition; and (c) the full utilization of appropriate and effective measures for its treatment and control.

The first of these—adequate and effective publicity—is now under way and plans for the effective mobilization and utilization of the available resources for the diagnosis and treatment of syphilis are now in process of formation.

Syphilis knows no social strata and Dr. Parran has therefore enunciated as a basic principle essential to the success of the

campaign, that facilities for the diagnosis and treatment of syphilis must be freely available to all, regardless of social or financial status.

There can be little question but the incidence, spread, and sequelae of syphilis are definitely influenced by inadequate treatment. Nor can it be denied that inadequate treatment may depend upon various factors, among which may be mentioned inadequate general training in the basic principles of the treatment of this disease; some degree of apathy on the part of health officers and, perhaps, of the medical profession in some degree as well; and, last but not least, the economic factor as concerns the patient.

While it is true that much difficulty is often experienced in persuading patients of the necessity for adequate and prolonged observation and treatment, it is also true that in no small measure the failure to continue treatment to an effective degree is often determined by the economic factor.

It follows, therefore, that for the campaign to be in full measure successful and effective, both diagnostic and therapeutic facilities must be freely available to all regardless of their ability or inability to pay for them; and this in turn necessitates that the expenditures involved must be borne by the Federal Government and the component states of the Union.

It is clearly realized that while the Federal Government and the State Governments may readily supply the drugs necessary for the treatment of syphilis, it is impossible for either or both to supply an adequate personnel for their administration. It is also appreciated that many individuals will prefer the services of their own physician regardless of what others may be offered.

It is apparent, therefore, that in this campaign the physician at large must play an important part and that his services must be sought for and utilized. It is also appreciated, however, that it would be unjust and impossible to demand that he administer treatment without some remuneration, even though the necessary drugs are supplied free of cost, for the intelligent and successful treatment of syphilis demands more than the ability to handle a syringe. Provisions are made, therefore, for the payment of the

physician taking part in the campaign. Whether the remuneration will be adequate and in keeping with the professional ability required is not as important as the recognition of the principle involved.

The first and essential requisite for the successful treatment of any disease, however, is the recognition of its existence. This is particularly true of syphilis, the diagnosis of which is often possible only through specialized laboratory procedures requiring both skill and extended experience. These procedures are also essential in the control of the disease as furnishing means—often the only means available—for the effective control of treatment. The importance and essential necessity for the participation and utilization of the laboratory and the clinical pathologist in this campaign is at once obvious. The clinical pathologist, may, in fact, be said to be the “key man” of the situation, for without his effective participation both diagnosis and adequate treatment will be seriously hampered if not gravely crippled.

It might be said—doubtless without due consideration of the problem as a whole—that the clinical pathologist might safely be ignored and replaced by expansion of state department of health laboratories.

Nothing can be further from the truth. Not only are state laboratory facilities utterly inadequate, both from the standpoint of number and location, but such laboratories may be lacking in the matter of skilled personnel.

The clinical pathologists on the other hand, form not only a strategically located network over the country at large, often functioning where state laboratory facilities are nonexistent or inaccessible, but bring to the problem not only technical ability, but the skill and experience of the specializing and specially trained physician. Few will deny that the interpretation of laboratory reports is of more significance than the report itself. This is especially true of serological reports and it is here that the services of the clinical pathologist are not infrequently of paramount value. The same situation, perhaps even in greater

degree, obtains in the all-important dark-field diagnosis of early syphilitic lesions.

It is quite true that state laboratory facilities could be expanded to a point where the existence of the clinical pathologist might be in jeopardy. It is equally true that the aftermath of any such policy would be doubly disastrous to medicine as a whole and the campaign against disease in general.

It is impossible to overemphasize this fact. When the existence of the clinical pathologist is jeopardized the deterioration of medicine as a whole has begun and will continue, inevitably and beyond question. For there must be clinical pathologists—physicians trained in the study of the mechanisms and causes of disease and trained in the study, recognition, and evaluation of their manifestations and aftermath—there must be clinical pathologists to enable the study, elucidation and control of disease; to assist the physician in his diagnostic problems and in the newer and highly developed biologic methods of treatment; and to supervise and direct the activities of clinical laboratories, whether under Government, state, municipal, hospital or private auspices.

Unless this specialty is jealously conserved, nurtured and maintained as an attractive and remunerative branch of the practice of medicine there will be no incentive for young physicians to embark upon it and no more clinical pathologists will be available when those now in the field have gone.

In such case, from whence will come the specialized students of disease, the investigators of the future, the laboratory consultants to whom, not infrequently, both physician and surgeon turn for aid and counsel?

*For no matter how extensive its equipment or how skilled its technical personnel, the fulfilment of the function of the clinical laboratory and its value to physician, patient, and community alike must stand or fall upon the reputation, skill, and ability of the clinical pathologist by whom it is directed.*

This is thoroughly understood and appreciated by the United States Public Health Service. Surgeon General Parran is definite

and emphatic in his desire that, in the necessary expansion of laboratory facilities incident to the syphilis campaign, the fullest possible use be made of existing private and hospital laboratories even to the point of stimulating by subsidy the establishment of private laboratories by clinical pathologists where none are now available and, by the withdrawal of existing state laboratory facilities, if necessary, to make the volume of work going to such laboratories sufficient to secure an adequate return.

In fact, it has been specifically said that "it is not deemed feasible or advisable to restrict the performance of blood serologic tests to a central state laboratory", and there is ample and concrete evidence of the desire of the Surgeon General to utilize the private laboratories to the fullest extent in the campaign against syphilis.

It may be stated with authority that the Public Health Service approves the extension of subventionary assistance to properly qualified laboratories when this work can be economically performed for medically indigent patients.

It must be appreciated, however, that the United States Public Health Service has no dictatorial power over the states. It may inaugurate a campaign against syphilis. It may offer Federal Funds for the conduct of such a campaign, as it has, providing such funds are matched by the state or community. It may suggest plans whereby such a campaign may be or should be conducted. It may upon request recommend and advise—but it cannot dictate.

Whether, for example, the clinical pathologist is to be utilized, subsidized, or ignored in a particular state does not and will not, depend upon the Public Health Service nor upon the recommendations of any society or organization. This will be governed entirely by the liaison and contact between the clinical pathologists of that state and the state health authorities engaged in formulating the campaign for that state.

It is imperative, therefore, that in each state and municipality contemplating participation in the campaign against syphilis the clinical pathologists of that locality consider among themselves the manner in which they may best and most adequately



coöperate; formulate the plan which they believe best, and determine the fairest, and most just allotment and distribution of the funds available for laboratory expenditure and then, with the approval, if necessary of county and state medical organizations, present and support it before the state authorities responsible for the inauguration and conduct of the campaign in that state.

Nor can the pathologist fail of clinical support if it be but recalled that the clinical pathologist, after all, is a physician specializing in a particular phase of the practice of medicine and that his fate in some measure foreshadows the future of medicine laas whoe.

If, as some maintain, the clinical pathologist stands at the crossroads, it is in large measure within his power to choose which path he will follow or, by adopting a passive and inert policy of *laissez faire*, to accept whatever the fates may decree.

R. A. K.

#### CAVEAT EMPTOR

It is essential, for various and somewhat obvious reasons, that the campaign against syphilis shall be heralded and attended by publicity and propoganda directed toward the public education. It is also essential, and directly related to the success of the campaign that such publicity be regulated—in so far as this may be possible—and that ill-advised, if not misleading statements avoided.

The danger is two-fold: first, that the campaign shall not be over-publicized and thus become only “a nine days’ wonder”, and, second, that the information disseminated shall be accurate and, in its implications, safe.

It is, perhaps, only a coincidence that a large commercial house is now pushing, through its detail men, a “diagnostic reagent for the rapid diagnosis of syphilis”.

In other words, this is the antigen for a flocculation test first described in 1935 on the basis of 400 blood specimens and 20 spinal fluid specimens. But one other paper has been published, in 1937, concerning this reaction which, it is fair to assume, has

yet to win its laurels and prove its value—as others have done—through extended comparative trial in the hands of numerous workers.

It is said, in the advertising literature of this test, that it is “especially adapted for use in small hospitals and clinics, and for emergency use”; that, “as it resembles the method employed in blood grouping, hospital internes and technicians are familiar with the technic”; that “the reagent is available at a moment’s notice.”

It is thus all very simple and easy. BUT—“many chemicals, such as acetic acid, interfere with the test. The operator must be alert and assume some measure (sic) of responsibility to insure the use of only sensitive, reliable, active test reagents.”

This seems to be rather a mild statement in view of the fact that the experienced and skilled serologist has always been prepared to assume—and has expected to assume, as a matter of course—not “some” but full responsibility for all phases of his serologic reactions, whether personally performed, or carried out under his supervision.

And, it may be said that, because of his experience and skill and his intimate acquaintance with the complexities of the serology of syphilis, he would be somewhat reluctant to accept the serologic diagnosis of the average hospital interne or even the average technician.

Moreover, as usual, the performance of the test in question requires meticulous attention to various essential details—as is true of all flocculation tests. In addition, the reagent must be carefully prepared (“activated”) by the operator with due care to avoid hypersensitivity.

In other words, this test, like all serologic tests in syphilis, requires care, skill and, for safe and reliable results, a thorough understanding of serology in general.

It is said that this procedure is at present being detailed only to hospitals. But it is permissible to speculate how soon it will be presented as a handy procedure for the physician’s office.

Indeed, another firm has for some time advertised a handy outfit for the performance of a flocculation test for \$9.75 all

complete—except, perhaps, for the skill and experience essential to escape disaster!

One wonders what the reply of the detail man—or the firm—would be to this question: “would you be willing to accept a diagnosis of syphilis with all that it implies, if made upon yourself or some member of your family based upon the results of this test in the hands of any customer to whom you have sold it?”

The question was asked—but the answer has yet to come.

R. A. K.



## THE OPPORTUNITIES OF THE CHEMISTRY DIVISION FOR SERVICE TO CLINICAL MEDICINE\*

F. WILLIAM SUNDERMAN

*William Pepper Laboratory of Clinical Medicine, Department of Research  
Medicine, University of Pennsylvania*

In assessing the opportunities for service presented to the chemistry divisions of our modern medical institutions it must be apparent that in addition to helping in the diagnosis, prognosis, and treatment of disease, one of the most important opportunities of the chemistry division lies in the *furtherance of the investigative viewpoint in medicine*. The expression "investigative viewpoint" is used to imply a desire to extend our knowledge of disease by analytical and quantitative methods and to connote an inquiring mode of thinking, a dissatisfaction with the superficial, and the avoidance of fixed ideas. Before such a society as this, further elucidation of the full meaning of the term, "investigative viewpoint," is unnecessary. Lord Moynihan regarded every surgical operation as an experiment and Sir William Jenner thought of the administration of every dose of a drug as a research problem. This type of approach to the problems of medicine, so largely developed by the science of chemistry, is, in a real sense, the cement which binds together the very foundations of modern medicine.

A plea for the investigative viewpoint, I am fully aware, requires no defense before you. To those, however, who would raise the question of the cost entailed in the development of research facilities it should be pointed out that there are many degrees of elaborateness in tackling research problems and that even routine laboratory analyses may be so conducted as to take on the character of research work. The comment of Dr. Alfred

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Stengel in a recent address that "a medical school unconcerned with research must be sterile indeed and lacking in all stimulus for its student body" applies equally to the hospital laboratory and its staff. In general, it would appear evident that the usefulness and stimulation which might be derived from a given hospital laboratory is bound up inextricably with the quality of its creative research and the breadth of its experimental point of view.

A clearer perspective of the present day outlook and trends in medical chemistry might better be gained by pausing briefly to survey the historical relationships of medicine to chemistry.

At the dawn of the modern scientific era no distinction was made between the work of the physician and the chemist. Even in the confused period of alchemical delusion, chemistry was cultivated almost exclusively by physicians. They considered it a "high and sacred art—an art proper to be known and practiced only by the pure, the benevolent, the learned, and the wise." The tyranny of superstition in this period was broken when Paracelsus, a physician and chemist, vigorously denounced the idea of transmutation of elements through processes of magic and necromancy and declared that the real purpose of chemistry "was not to make gold but to prepare medicines." However, to Robert Boyle, living in the latter part of the seventeenth century, rightfully belongs the title of first modern biochemist. It was he who substituted and advocated the experimental methods of chemistry in place of the descriptive Aristotelian concepts whose adherents he stigmatized as "propounders of riddles and impertinences." In his "Memoirs for the Natural History of Human Blood," published in London in 1684, he directed attention to the stagnant nature of the descriptive method when in the preface of the book he stated:

"I willingly acknowledge that divers physicians have amply and learnedly and some of them very eloquently, set forth the praises of the blood, and manifested how noble and excellent a liquor it is. But I must beg their pardon if I doubt whether their writings have not celebrated the praises, than discovered to us its nature."

During the latter part of the eighteenth and early part of the nineteenth century, chemistry, as a subject of study in a



university, was considered merely as a branch of medicine—the professor of medicine not infrequently holding both chairs. The physician-chemists of this period constituted a distinguished group of individuals, including such men as William Cullen, professor of medicine and chemistry of the Universities of Glasgow and Edinburgh; Joseph Black, inventor of the analytical balance and renowned for his celebrated contributions to the subject of heat; William Prout, famous for the well-known hypothesis which bears his name and for the discovery of free hydrochloric acid in the gastric juice; and many others of eminence who worked in the dual capacity of chemist and physician. Among the pupils of Joseph Black was one Benjamin Rush, a native of Philadelphia, who upon his return to this country became at the University of Pennsylvania the first professor of chemistry in America. During this period Lavoisier sounded the death knell for the phlogiston theory by demonstrating that both breathing and combustion were oxidative processes.

Soon afterwards interest in organic chemistry was awakened when Wöhler doomed the current vitalistic philosophy by proving that urea could be produced entirely from inorganic matter. Within the same period Serturner's isolation of morphine served as the stimulus which led within a couple of decades to the isolation of strychnine, brucine, quinine, atropine, and other alkaloids. New hope for organic chemistry was thus offered and Wöhler's epoch-making discovery may be regarded as having initiated that brilliant series of organic syntheses reaching a pinnacle at the end of the century in the illustrious work of Emil Fischer. Four years after Wöhler's discovery, Liebig, in 1832, synthesized chloral hydrate, the first synthetic chemical compound that was shown to have therapeutic value.

The middle of the past century found the physician-chemists engaged not only in the synthesis of new compounds for therapeutic use, but also in the analysis of the end-products of body metabolism. Thus, out of the organic chemistry of the period was developed that branch of chemistry which we now call physiological chemistry. In the beginning the efforts of the physiological chemists were spent largely in the development of qualitative methods of analysis. Indeed, many of the common

qualitative or semi-quantitative color tests that are now in daily use were developed during this period. Under the stimulus of von Voit and von Pettenkofer in Germany emphasis was directed to the physical and chemical considerations of physiological processes. The new science of thermodynamics, being incorporated into physiology, led to the expression of metabolism in energy terms. In France the study of the chemistry of pathological conditions was advocated by Claude Bernard who boldly asserted that his chief aim was to "make the well-known principles of the experimental method pervade medical science."

At the beginning of this century internal medicine had developed to the point where chemical help was indispensable in the diagnosis and treatment of the patient. Out of this need was envolved the present biochemistry division of the hospital laboratory. Initially, this branch of chemistry utilized the older methods of physiological, inorganic, and physical-chemistry but it soon was compelled to develop its own especial procedures for the study of disease observed in the hospital and clinic. Noteworthy was the remarkable development of micro-methods of analysis which rapidly replaced more cumbersome macro ones. The orthodox chemist was first prone to look with derision upon micro analyses and to dismiss them as "pretentious types of cookery"; later however, he recognized their value and importance in fields outside of medicine and assisted materially in their development.

An opportunity for service clearly exists in the development of even more simple, rapid, and accurate methods of analysis. In many instances the usefulness of a given method depends upon the time and labor expended and upon the promptness with which the results of an analysis are made available. It can be argued that scientific progress may depend in large measure upon the development of new methods of analysis yielding greater accuracy, specificity, simplicity, or speed. One might well ponder, for instance, whether or not our knowledge of the use of insulin or, perhaps, even its discovery, could have been possible without the timely improvements in the methods for the analysis of blood sugar. In this respect the words of Lord Kelvin would

seem appropriate, "When you can measure what you are speaking about and express it in numbers, you know something about it; when you can not express it in numbers, your knowledge is of a meagre and unsatisfactory kind."

While it is obviously difficult to predict the character which biochemistry may assume in the future, nevertheless, it is possible to point at least to one direction in which it appears to be advancing. This has been indicated to some extent by Cathcart and termed by him "dynamic biochemistry." The newer development would appear to be inclined toward the application of chemical and physical methods directly to the living organism and to the observation of changes occurring within living cells. Many examples of recent investigations showing this tendency might be cited, such as measurements of changes occurring in the various fluid compartments in the body; measurements of various types of clearances; direct measurements of the products of intermediary metabolism of surviving isolated tissue by means of the Warburg apparatus, et cetera. One striking example that gives promise of value in the clinic is the measurement of the total content of a given component in the blood. At present there is a fairly good comprehension of the changes in concentration of the various components of the blood that may occur in given pathological conditions. However, what is known about the changes in the total content of these components? Practically nothing! To obtain measurements of the total content of a component, the determination of blood volume is required. All of these types of measurement necessitate an intimate and responsible relation of the chemist to the patient. It would thus seem desirable that to pursue this trend the biochemist should have competent clinical experience.

The newer biochemistry can not restrict itself entirely to the older problems pertaining to metabolism, chemical structure, and the application of physical chemical laws to protein-containing solutions. The importance of further elucidation of these problems is acknowledged to be essential. However, in addition to determining whether an OH group in a sterol compound is in the ortho or para position, one of the responsibilities of the newer

biochemistry is clearly in the evaluation of the function and importance of this grouping to the living organism by means of the methods of experimental medicine. Lord Moynihan had this thought in mind when, in an address a few years ago, he stated:

"I believe that the recent, so confident success of laboratory work owes much to the original impulse derived from clinical work, that when left to himself the laboratory worker has gone a little astray, and that, freed from the final need for clinical verification his efforts, admirable in themselves, have in no small degree been wasted so far as medicine is concerned."

An important responsibility of the biochemistry laboratory is in the training of practicing physicians, internes, and technicians. It almost goes without saying that an individual who has learned for himself a method of analysis is, as a consequence, better able to judge of its value and significance. To develop competency and precision in performing analyses technicians usually require long training and continued supervision. To quote the late Otto Folin, "Every method goes wrong now and then, and the physician who is as helpless as the technician when something does seem to be wrong has but meager qualifications for the work he is trying to do."

An opportunity for service clearly exists in the complete cooperation of the biochemistry division with the other divisions of the hospital laboratory. For example, it would seem not too much to hope that eventually the chemical isolation of the active constituents of vaccines and serums will be accomplished; already the molecular weights of antibodies are being reported. The necessity for team-work and coordination of the laboratory divisions is self-evident.

An opportunity would be neglected if I failed to mention the need for adequate supervision of commercial clinical laboratories. Practicing physicians frequently lack critical judgment in evaluating the results of chemical analyses. I believe it to be a necessary development of the future that such commercial laboratories be required to maintain a high degree of accuracy and standard of responsibility in their dealings with practicing physicians and the public.

Finally, to those individuals who pessimistically foresee our hospital laboratories and medical schools pervaded by ultra scientific, soulless, coldly rationalistic individuals without that much discussed and elusive attribute, the so called art of medical practice, might I reply with Billroth's words, spoken a half a century ago, "It is one of the superficialities of our age to see in science and in art two opposites; imagination is the mother of both."

## THE ONE-HOUR TWO-DOSE GLUCOSE TOLERANCE TEST\*†

S. E. GOULD

*From the Departments of Pathology, Wayne University College of Medicine, Detroit, and Eloise Hospital and Infirmary, Eloise, Michigan*

In the ordinary three-hour glucose tolerance test, one often encounters equivocal curves which may be interpreted as either diabetic or non-diabetic. Such doubtful or diabetic curves are particularly frequent among aged or arteriosclerotic individuals<sup>1, 2, 3, 4</sup>, and may also be found in other non-diabetic conditions<sup>5</sup>. The six-hour two-dose test<sup>6</sup>, which attempts to obviate this shortcoming, is based upon Allen's law that the greater the amount of sugar given, the more will be utilized in the normal individual, while the reverse is true in the diabetic. Exton and Rose<sup>7</sup> made use of this law in introducing a one-hour two-dose test which has the advantages over the six-hour two-dose test in being shorter and in necessitating fewer venous punctures and less laboratory work.

In a previous communication<sup>4</sup> the one-hour two-dose glucose tolerance test proposed by Exton and Rose was compared with the three-hour test and the six-hour test among 45 individuals. The purpose of the present paper is to compare the results of these three tests in another series of 215 individuals.

### METHOD

Two hundred fifteen patients, including many known diabetics, were first given the one-hour test and after three to seven days, the six-hour test. In the one-hour two-dose test Exton and Rose's method was modified by administering 1.75 grams of glucose per kilogram of body weight in a 40 per cent solution instead of 100 grams dissolved in 650 cc. of water. One-half of the

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† Because of lack of space, this article is abbreviated. The complete article appears in the author's reprints.



calculated amount was given after the fasting specimens of blood and urine were taken and one-half was given after the one-half-hour specimens were taken. In the six-hour two-dose test 1.75 grams of glucose per kilogram of body weight (or one-half of this amount) was given both after the fasting and after the three-hour specimens were collected. In this study, for the purposes of comparison, the first portion of the six-hour curve (up to the end of the third-hour period) was used as the three-hour curve. The Folin-Wu method of glucose determination was used.

The results obtained with the six-hour test were used as the standard for comparison and the curves were classified as "diabetic" (D) or "non-diabetic" (-). The criteria adopted for the presence or absence of diabetes mellitus in the various tests are as follows:

#### 1. Three-hour test

"Normal" (-). The venous blood sugar at one hour is below 170 mgm., at two hours below 120 mgm., and at three hours has returned to the fasting level.

"Diabetic" (D). The venous blood sugar at one hour is 170 mgm. or over, at two hours 120 mgm. or over, and at three hours 10 mgm. or more above the fasting level.

"Borderline" (?). The levels at one or two hours are above normal and the third hour value is 10 mgm. or more above the fasting level.

#### 2. Six-hour two-dose test

"Non-diabetic" (-). The curve at three hours is normal or borderline, and at six hours has returned to the fasting level.

"Diabetic" (D). The curve at three hours is borderline or diabetic, and at six hours is 10 mgm. or more above the fasting level.

#### 3. One-hour two-dose test

a. *Criteria of Exton and Rose.* "Normal" (-). The fasting venous blood level is normal, the one-half hour value is not over 75 mgm. above the fasting reading, the one-hour value is not over 5 mgm. above the one-half reading, and there is no glycosuria in any of the specimens.

"Diabetic" (D). The one hour level is 10 mgm. or more above the half-hour level and the reading is 170 mgm. or higher (a blood sugar level of 170 mgm. or over was used in lieu of glycosuria since in many individuals the urine specimen could not be obtained).

b. *Criteria of Gould, Altshuler and Mellen.* "Normal" (-). The fasting blood sugar is less than 120 mgm., the half-hour level is less than 50 mgm. above the fasting value, and the level at one hour is less than 30 mgm. above the half-hour value.

"Diabetic" (D). The presence of any two of the following three conditions

indicates diabetes: (1) a fasting blood sugar of 120 mgm. or over; (2) a one-half-hour level of 50 mgm. or more above the fasting value; and (3) a one-hour level of 30 mgm. or more above the half-hour value.

### RESULTS

There were 143 "non-diabetic" and 72 "diabetic" six-hour curves. Among the individuals with "non-diabetic" six-hour curves (tables 1 and 2) 74 were aged up to 40 years. In these

TABLE 1  
SHOWING INTERPRETATION OF VARIOUS GLUCOSE TOLERANCE CURVES AMONG 143  
INDIVIDUALS WITH SIX-HOUR "NON-DIABETIC" CURVES

	3-HOUR TEST	6-HOUR 2-DOSE TEST	1-HOUR 2-DOSE TEST		AGE GROUPS			
			Exton and Rose	Gould, Altshuler and Mellen	Up to 40	47 to 59	60 and over	All groups
	—	—	—	—	48	3	14	65
	?	—	—	—	7	4	18	29
	—	—	—	D	1			1
	—	—	D	—	2	2	3	7
	—	—	D	D	3	1	1	5
	?	—	D	—	3	1	5	9
	?	—	D	D	2	1	3	6
	D	—	—	—	2		2	4
	D	—	D	—			4	4
	D	—	—	D			1	1
	D	—	D	D	6	2	4	12
Totals					74	14	55	143
?	44							
D	21		43	25				
—	78	143	100	118				

74 individuals, the three-hour test was "borderline" or "diabetic" in 20 instances, while the one-hour test gave 16 "diabetic" curves by the criteria of Exton and Rose and 12 "diabetic" curves by the criteria of Gould, Altshuler and Mellen. There were 69 individuals aged 47 years or over with "non-diabetic" six-hour curves in whom the three-hour test was "borderline" or "diabetic" in 45 instances, while the one-hour test gave 27 "diabetic" curves by the criteria of Exton and Rose and 13 "diabetic" curves by the criteria of Gould, Altshuler and Mellen.

In table 3 is shown the interpretation of various glucose tolerance curves among 72 individuals of all ages with "diabetic" six-hour curves. The one-hour test gave "non-diabetic" readings in 6 instances in which the standards of Exton and Rose were

TABLE 2

COMPARISON OF 143 "NON-DIABETIC" SIX-HOUR TESTS WITH RESULTS OF THREE-HOUR AND ONE-HOUR TWO-DOSE TESTS

TESTS	RESULTS	AGES UP TO 40	AGES 47 TO 59	AGES 60 AND OVER	TOTAL
3-hour	Negative —	54	6	18	78
	Borderline ?	12	6	26	44
	Diabetic D	8	2	11	21
1-hour 2-dose (Exton and Rose)	Negative —	58	7	35	100
	Diabetic D	16	7	20	43
1-hour 2-dose (Gould, Alt- shuler and Mellen)	Negative —	62	10	46	118
	Diabetic D	12	4	9	25
Total .....		74	14	55	143

TABLE 3

SHOWING INTERPRETATION OF VARIOUS GLUCOSE TOLERANCE CURVES AMONG 72 INDIVIDUALS WITH SIX-HOUR "DIABETIC" CURVES

	THREE-HOUR TEST	SIX-HOUR TWO- DOSE TEST	ONE-HOUR TWO-DOSE TEST		TOTAL
			Exton and Rose	Gould, Altshuler and Mellen	
	?	D	D	D	1
	D	D	—	—	4
	D	D	—	D	1
	D	D	D	—	3
	D	D	D	D	63
Total { — ? D	1		5	7	
	71	72	67	65	

used and in 7 instances in which the criteria of Gould, Altshuler and Mellen were applied. In these 72 "diabetic" six-hour curves, the fasting blood sugar was less than 120 mgm. per cent in 41 instances and 120 mgm. per cent or over in 31 instances.

In table 4 is listed the interpretation of the six-hour and the one-hour two-dose tests. It will be seen that among the "non-diabetic" six-hour curves, the one-hour test was called "diabetic" in 20 instances according to the standards of Exton and Rose only; in 2 instances according to the standards of Gould, Altshuler and Mellen only; and in 23 additional instances according to the criteria of both of these groups of workers. Among the "diabetic" six-hour curves, the one-hour test in 1 was interpreted

TABLE 4  
COMPARISON OF SIX-HOUR TWO-DOSE TEST WITH ONE-HOUR TWO-DOSE TEST  
ACCORDING TO CRITERIA OF EXTON AND ROSE (ER) AND OF GOULD, ALTSHULER  
AND MELLEN (GAM)

D indicates "diabetic", - indicates "non-diabetic"

	6-HOUR TEST	1-HOUR 2-DOSE TEST		TOTAL
		ER	GAM	
143 "non-diabetic" 6-hour curves	-	-	-	98
	-	D	-	20
	-	-	D	2
	-	D	D	23
72 "diabetic" 6-hour curves	D	-	-	4
	D	-	D	1
	D	D	-	3
	D	D	D	64

as "non-diabetic" by the criteria of Exton and Rose only, in 3 by the criteria of Gould, Altshuler and Mellen only, and in 4 as "non-diabetic" by the standards of both groups of workers.

#### COMMENT

Inasmuch as the oral glucose tolerance test may, in some instances, fail to detect, and, in other instances, fail to exclude the presence of diabetes mellitus<sup>3</sup>, the particular test used may have to be interpreted in conjunction with the clinical findings of the patient. Among the individuals with "non-diabetic" six hour curves, there were a number with diseases in which the three-hour curves are often "borderline" or "diabetic." There were 7 individuals clinically diagnosed as having diabetes mellitus,

and 7 individuals with essential hypertension, all of whom had three-hour curves that were "borderline" or "diabetic." All 14 of these individuals had one-hour curves that were "diabetic" by the criteria of Exton and Rose, and 10 of them (5 in each group) were "diabetic" by the criteria of Gould, Altshuler and Mellen. Among the older individuals, aged 47 and over, with "non-diabetic" curves, there were 45 with "borderline" or "diabetic" three-hour curves and 13 with "diabetic" one-hour curves as judged by the standards of Gould, Altshuler and Mellen. Eleven of these 13 curves occurred in individuals with diseases, other than arteriosclerosis, which commonly have an abnormal glucose tolerance curve and only 2 in individuals with arteriosclerosis alone. In other words, in the large majority of older (arteriosclerotic) individuals in whom the three-hour test is "borderline" or "diabetic," the one-hour two-dose test will rule out diabetes, provided the criteria of Gould, Altshuler and Mellen are applied. An analysis of the curves will show that the latter criteria ruled out diabetes in 30 of 32 of these older patients in whom the three-hour test was "borderline" or "diabetic."

Among the 72 "diabetic" six-hour curves there were no "non-diabetic" three-hour readings, since one of the criteria for a six-hour "diabetic" curve stipulated that the reading at the three-hour period be either "borderline" or "diabetic." In all of the instances in which the one-hour two-dose test gave "non-diabetic" curves, the fasting blood sugar was less than 120 mgm. per cent and the diabetes as judged by the six-hour test would be considered as of mild type. In 5 of the 7 instances in which the one-hour test was interpreted as "non-diabetic" by the criteria of Gould, Altshuler and Mellen, the patient clinically was not considered to have diabetes mellitus.

In the three-hour test, the diagnosis of diabetes is based upon an abnormal blood sugar peak and a delayed fall in the sugar level; in the six-hour test, the second peak is normally lower than the first peak<sup>4</sup> and at the sixth hour the blood sugar has returned to, or fallen below, the fasting level. The one-hour test indicates the body's response to a second dose of glucose thirty minutes after its ingestion, but gives no clue as to the subsequent rate of

glucose utilization. An analysis of the curves shows that many of the one-hour and three-hour "diabetic" curves are found among individuals listed as "non-diabetic" by the six-hour test because in the latter test the blood sugar level has returned to or below the fasting level at the fifth or sixth hour. This failure of the one-hour two-dose test to indicate the rate of glucose utilization after the sixty-minute period, appears to be a serious shortcoming in many instances.

#### SUMMARY

A comparison of the three-hour, the six-hour two-dose, and the one-hour two-dose oral glucose tolerance tests was made among 215 patients. Using the six-hour two-dose test as the standard for comparison, 143 curves were found to be "non-diabetic" and 72 "diabetic." The one-hour two-dose test of Exton and Rose was found to be superior to the three hour test, particularly if the criteria of Gould, Altshuler and Mellen are applied. This superiority of the one-hour test was especially evident in the diagnosis or exclusion of diabetes mellitus among arteriosclerotic patients.

An inadequacy of the one-hour two-dose test appears to lie in its failure to indicate the rate of glucose utilization after the sixty-minute period.

The author wishes to acknowledge his appreciation for the painstaking technical assistance rendered by Miss Grace Kercher and Dr. Emeline Freeborn.

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## OBSERVATIONS ON THE ONE HOUR TWO DOSE DEXTROSE TOLERANCE TEST\*

J. SHIRLEY SWEENEY, J. J. MUIRHEAD AND LOUIE E. ALLDAY

*Dallas, Texas*

Recently Exton and Rose<sup>1</sup> published results of what they termed the "one-hour two-dose dextrose tolerance test", an ingenious modification of the ordinary dextrose tolerance test originally designed to obviate some of the misleading results obtained by the more commonly used procedures. They mentioned various factors influencing the commonly employed dextrose tolerance test—the effects of antecedent diets or food habits of the individual examined, the time the test was performed, rate of absorption from the intestine of the ingested dextrose, and so on. On the basis of the paradoxical law of dextrose,<sup>†</sup> after varying the experimental conditions under which repeated tolerance tests were made, these investigators conceived the idea of giving two doses of glucose 30 minutes apart, on the assumption that the first dose would activate the insulin glycogen mechanism. The authors state that this test "gave no evidence of being in any way affected by antecedent diets or irregularities of absorption, and very definitely indicated the practicability of adopting the paradoxical law to routine clinical use." The value of the Exton two-dose one-hour test has been thoroughly proved. There is no question but that it eliminates many abnormal tolerance curves, more especially those showing an exceptionally high

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† "The paradoxical law of dextrose distinguishes sharply between diabetic and every type of non-diabetic animals. . . . Limits of tolerance in non-diabetic animals are all apparent, not real. The limits of tolerance in diabetic animals are real, not apparent; just the opposite of the paradoxical law. . . . Whereas in normal individuals the more sugar given the more is utilized, the reverse is true in diabetes" (F. M. Allen).

rise at the end of one hour, even though the blood sugar returns to a normal level at the end of two hours. Such curves have always been difficult to evaluate and it is just in these border line cases that the two-dose one-hour test has its greatest value and undoubtedly will become a more dependable means of differentiating suspected and renal diabetes from true diabetes. The

TABLE 1

RESULTS OF THE ONE-HOUR TWO-DOSE TOLERANCE TESTS ON FOUR NORMAL YOUNG ADULTS WHO HAD BEEN ON AN EXCLUSIVE PROTEIN DIET FOR TWO DAYS PRIOR TO TESTS

SUBJECT	FASTING	30 MINUTES	60 MINUTES	90 MINUTES	120 MINUTES
A	65	155	170	165	142
B	70	160	190	200	182
C	64	133	148	138	121
D	70	127	143	143	108

TABLE 2

RESULTS OF THE ONE-HOUR TWO-DOSE TOLERANCE TESTS ON SIX NORMAL YOUNG ADULTS WHO HAD BEEN ON AN EXCLUSIVE FAT DIET TWO DAYS PRIOR TO TESTS

SUBJECT	FASTING	30 MINUTES	60 MINUTES	90 MINUTES	120 MINUTES
A	60	155	400	350	275
B	95	177	240	190	145
C	70	90	154	158	143
D*	69	125	103	103	111
E	64	87	190		174
F	69	121	174		106

\* This is a queer curve. In all probability absorptive factors were responsible for its distortion.

test, also, has the advantage of requiring less time for its execution, and is certainly less disturbing to the patient.

The present study was made to determine the effects of extremely abnormal antecedent diets on the two-dose one-hour test. We<sup>2</sup> have shown previously that healthy, normal young adults who had fasted for 48 hours, and who had been on exclusive high fat diets as well as exclusive protein diets for a period of 48 hours preceding the ordinary dextrose tolerance test, yielded

typical diabetic curves when given 1.75 grams glucose per kilogram of body weight. This was particularly true of those who had fasted and had been on fats.

Normal, healthy, young adults were used in the present study. Some of the students abstained from all food for forty-eight hours,

TABLE 3

RESULTS OF THE ONE-HOUR TWO-DOSE TOLERANCE TESTS ON FOUR NORMAL YOUNG ADULTS WHO HAD EATEN NO FOOD FOR TWO DAYS PRIOR TO TESTS

SUBJECT	FASTING	30 MINUTES	60 MINUTES	90 MINUTES	120 MINUTES
A	69	105	157	177	184
B	52	114	181	221	210
C	67	111	156		182
D	67	108	190		217

TABLE 4

RESULTS OF THE ONE-HOUR TWO-DOSE TOLERANCE TESTS ON TWO NORMAL YOUNG ADULTS WHO HAD BEEN ON AN EXCLUSIVE CARBOHYDRATE DIET FOR TWO DAYS PRIOR TO TESTS

SUBJECT	FASTING	30 MINUTES	60 MINUTES	90 MINUTES	120 MINUTES
A	93	114	111	93	119
B	85	105	118	101	87

TABLE 5

COMPOSITE RESULTS OF THE ONE-HOUR TWO-DOSE TOLERANCE TEST ON NORMAL INDIVIDUALS WHO HAD BEEN ON EXCLUSIVE ANTECEDENT DIETS AS INDICATED IN TABLE

	FASTING	30 MINUTES	60 MINUTES	90 MINUTES	120 MINUTES
Proteins . . . . .	67	144	162	161	138
Fats . . . . .	71	125	210	200	159
Carbohydrates . . . . .	89	109	114	97	103
Starvation . . . . .	63	109	166	199	198

some ate only fats (egg yolks, cream and butter), some consumed only protein (egg whites and meat), while others ate only sugars for this period preceding the one-hour two-dose tolerance test.

The tolerance tests were performed according to the technic

outlined by Exton and Rose.<sup>1</sup> All blood sugar determinations were done by the Folin-Wu technic.

In tables 1 to 4, are listed the results of the tests in these different groups. In table 5, are the average figures for each group,

TABLE 6

RESULTS OF THE ONE-HOUR TWO-DOSE TOLERANCE TEST ON NORMAL INDIVIDUALS WHO HAD BEEN ON A GENERAL DIET TWO DAYS PRIOR TO TESTS

SUBJECT	FASTING	30 MINUTES	60 MINUTES	90 MINUTES	120 MINUTES
A	91	143	125	132	148
B	91	129	103	108	106
C	90	118	124	121	110

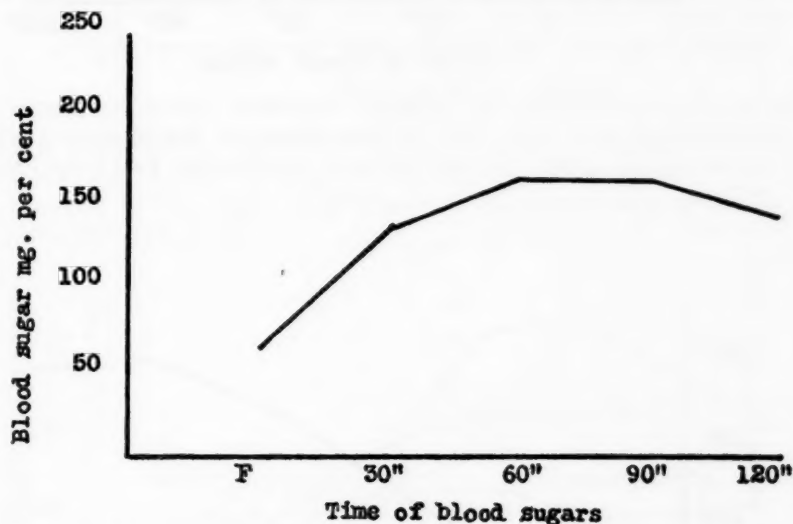


CHART 1. COMPOSITE GRAPH SHOWING ONE-HOUR TWO-DOSE DEXTROSE TOLERANCE CURVE (EXTENDED TO TWO HOURS) OF FOUR NORMAL YOUNG ADULTS WHO HAD BEEN ON AN EXCLUSIVE PROTEIN DIET FOR TWO DAYS PRIOR TO TESTS

while in table 6 are the results for the controls (those who were eating normally preceding the test). The results of these respective tests are presented graphically in charts 1 to 6.

Inspection of these figures and graphs reveals very definitely and conclusively that such extreme and abnormal antecedent

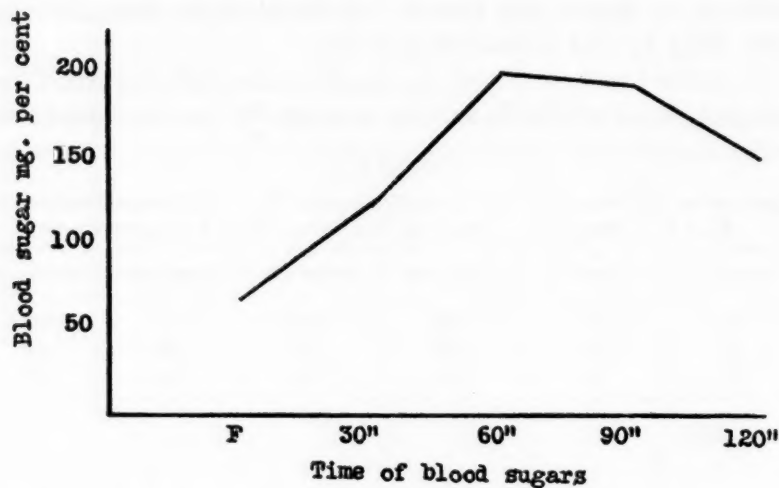


CHART 2. COMPOSITE GRAPH SHOWING ONE-HOUR TWO-DOSE DEXTROSE TOLERANCE CURVE (EXTENDED TO TWO HOURS) OF SIX NORMAL YOUNG ADULTS WHO HAD BEEN ON AN EXCLUSIVE FAT DIET FOR TWO DAYS PRIOR TO TESTS

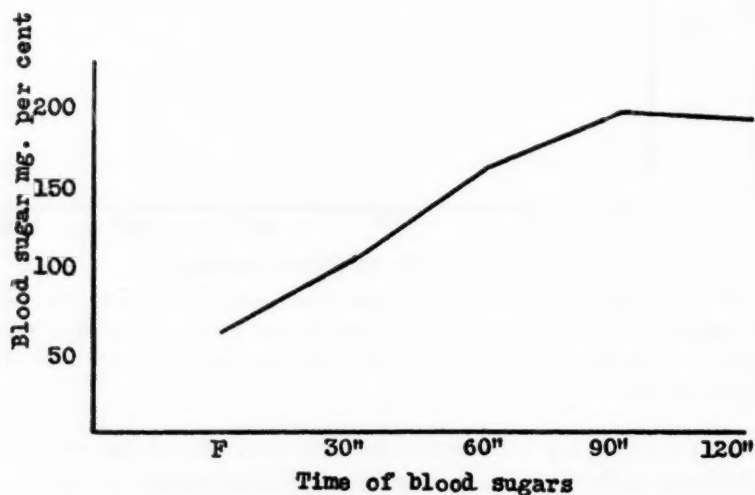


CHART 3. COMPOSITE GRAPH SHOWING ONE-HOUR TWO-DOSE DEXTROSE TOLERANCE CURVE (EXTENDED TO TWO HOURS) OF FOUR NORMAL YOUNG ADULTS WHO HAD EATEN NO FOOD FOR TWO DAYS PRIOR TO TESTS



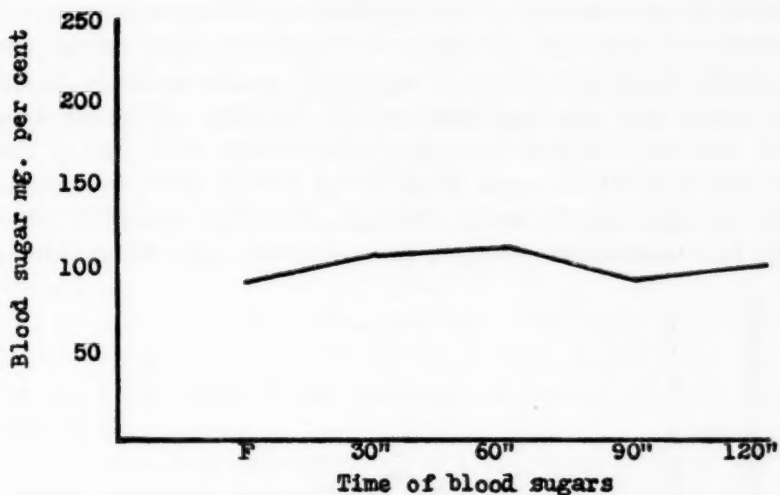


CHART 4. COMPOSITE GRAPH SHOWING ONE-HOUR TWO-DOSE DEXTROSE TOLERANCE CURVE (EXTENDED TO TWO-HOURS) OF TWO NORMAL YOUNG ADULTS WHO HAD BEEN ON AN EXCLUSIVE CARBOHYDRATE DIET FOR TWO DAYS PRIOR TO TESTS

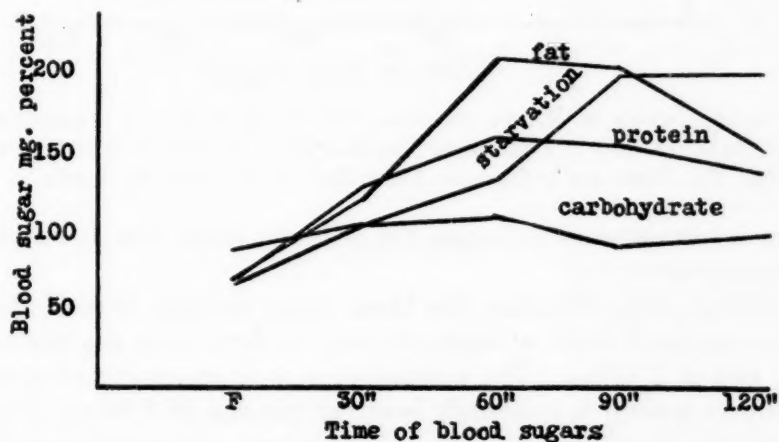


CHART 5. COMPOSITE GRAPHS SHOWING ONE-HOUR TWO-DOSE DEXTROSE TOLERANCE CURVES (EXTENDED TO TWO HOURS) OF NORMAL INDIVIDUALS WHO HAD BEEN ON DIFFERENT ANTECEDENT DIETS AS INDICATED

(See table 5)

dietary programs have a marked effect on the one-hour two-dose glucose tolerance test. In other words, the resultant curves were typically those of a diabetic, especially in the individuals who had fasted and who had been on high fat diets. There is, however, one notable and interesting observation, and that is that the one hour blood sugar levels in all groups were, in most instances, significantly lower, though abnormal (except in those who had received only sugars preceding the test), than the one

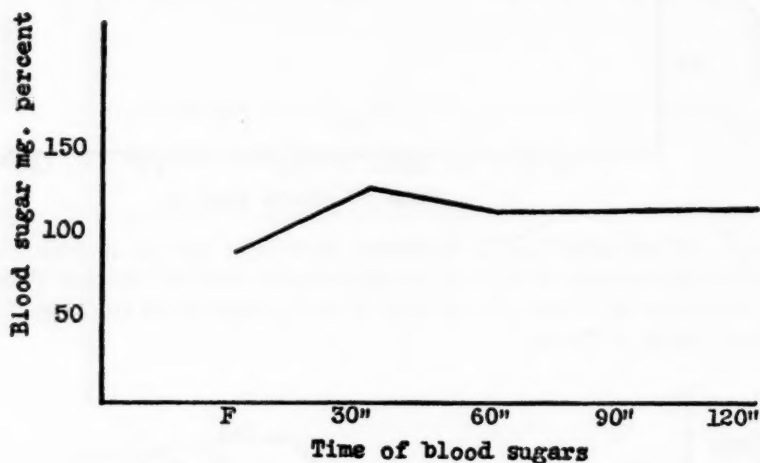


CHART 6. COMPOSITE GRAPH SHOWING ONE-HOUR TWO-DOSE DEXTROSE TOLERANCE CURVE (EXTENDED TO TWO HOURS) OF NORMAL YOUNG ADULTS WHO HAD BEEN ON A GENERAL DIET TWO DAYS PRIOR TO TESTS

hour levels obtained following the ordinary single dose dextrose tolerance test.<sup>2</sup>

Furthermore, following the blood sugar changes beyond the one hour limit, most of them are seen to drop more sharply at the end of 2 hours. The composite, or average, curve of each group in general is materially lower at the end of 2 hours than those following the single dose tolerance test. It is only reasonable to assume that these variations are due to the two doses of dextrose ingested at 30-minute intervals. The curves of the individuals used as controls, in general, were strikingly comparable to those presented by Exton and Rose,<sup>1</sup> and others who

have substantiated their findings. The two-dose one-hour test does show definite changes in individuals who have been on ordinary diets preceding the test. It is our opinion that it will serve as a very valuable differential diagnostic test.

#### SUMMARY

The effect of extreme and abnormal antecedent diets on the one-hour two-dose tolerance test of Exton and Rose<sup>1</sup> has been studied.

It is shown that such abnormal antecedent dietary programs distort the one-hour curves following 2 doses of dextrose in much the same way they do the ordinary, or commonly used, single dose tolerance test.

There is a slight tendency for the blood sugar levels not to rise to quite such high figures, and to return to relatively less abnormal values at the end of two hours.

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## CONTROL ANTIGEN EMULSIONS FOR MICROSCOPIC SLIDE PRECIPITATION TESTS FOR SYPHILIS\*

B. S. KLINE

*Laboratory Department, Mount Sinai Hospital, Cleveland, Ohio*

Control emulsions for the Microscopic Slide Precipitation Tests for the diagnosis and exclusion of syphilis with heated serum are described below. The control emulsion for the diagnostic test gives somewhat less sensitive results than does the standard diagnostic test emulsion and serves as an excellent control of the specificity of that emulsion. The control emulsions for the exclusion test give more sensitive though less specific results and more surely exclude syphilis than does the standard exclusion test emulsion.

### CONTROL EMULSION FOR THE SLIDE TEST FOR THE DIAGNOSIS OF SYPHILIS WITH HEATED SERUM

Since the introduction of the slide tests for the diagnosis and exclusion of syphilis with heated serum<sup>1</sup> there have been reports concerning them from various laboratories. The test for the diagnosis of syphilis has been reported upon more frequently than has the exclusion test, and in the majority of cases was found to give results of satisfactory specificity and sensitivity.<sup>2</sup> Some laboratories, however, have reported results with the diagnostic test of satisfactory specificity but of insufficient sensitivity<sup>3</sup> and still others have reported the sensitivity as satisfactory but the specificity as not maximal.<sup>4</sup> Likewise, as shown in the second series of the national evaluation of tests for the serodiagnosis of syphilis, the results of the diagnostic slide test as well as of all the other tests evaluated varied, at times considerably, in different laboratories.<sup>5</sup> The variation in results may be ex-

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plained by the differences in the quality of the antigen, cholesterolin, water, salt, and absolute alcohol employed and by the difference in the performance of the various steps of the technic.

For those laboratories getting unsatisfactory results with the diagnostic slide test it is advisable to make sure that the various ingredients are of standard quality and that the test is performed exactly as outlined. Even when the greatest care is exercised in the use of standard ingredients and in the performance of the test there is always some variation in the quality of the reagents employed at the same time in different laboratories and at different times in the same laboratory. Furthermore, in the preparation of the emulsions and in the results obtained, there is some variation not only by workers in different laboratories using the same ingredients but also by different workers in the same laboratory using the same ingredients.

For those laboratories getting more sensitive and somewhat less specific results than standard, and for those using the slide tests alone in the serodiagnosis of syphilis, it is advisable to perform a control diagnostic test with an emulsion containing less cholesterolin than standard. As reported previously, the sensitivity of the slide tests depends more upon the quantity of cholesterolin than upon the quantity of standard antigen present.<sup>6</sup> On the other hand, improperly prepared antigens themselves contain adventitious substances that increase the sensitivity of the emulsion. For instance, antigens prepared as for the slide tests but with 95 per cent ethyl alcohol in place of absolute alcohol, contain so much adventitious material that diagnostic test emulsions prepared with them give results so much more sensitive than standard and with so many false positive reactions that they are unsatisfactory for use. Such an antigen and about half the quantity of cholesterolin contained in the standard diagnostic test emulsion gives results about equal in sensitivity to those of the latter. Even with such a small quantity of cholesterolin, the improperly prepared slide test antigens give too many non-specific reactions. This emphasizes the fact that emulsions with poor antigen cannot be adjusted to give results as satisfactory as obtainable with emulsions of standard quality.

## PROTOCOL 1

*Diagnostic slide tests in cases of syphilis*

DATE	PATIENT	STANDARD EMULSION (1 CC. CHOLESTERIN SOLUTION)	CONTROL EMULSION (0.8 CC. CHOLESTERIN SOLUTION)
1937			
February 22 .....	F. G.	++++	+++
February 22 .....	C. B.	++++	++
February 22 .....	N. A.	++++	++
February 22 .....	E. S.	++++	+
February 22 .....	B. S.	+++	+
February 22 .....	L. M.	+	±
February 22 .....	N. K.	+	±
February 22 .....	D. T.	±	±
February 22 .....	R. O.	±	-
March 9 .....	G. C.	++++	++
March 9 .....	A. H.	++++	++
March 9 .....	P. C.	+	±
March 9 .....	M. B.	+	±
March 9 .....	S. T.	+	+
March 9 .....	S. G.	±	±
March 10 .....	V. W.	+++	+
March 10 .....	W. L.	+++	+
March 10 .....	B. B.	++	+
March 10 .....	I. S.	+	±
March 10 .....	C. L.	+	-
March 10 .....	C. S.	±	-
March 10 .....	L. M.	±	±
March 13 .....	W. B.	+++	+
March 13 .....	A. A.	+++	±
March 13 .....	R. B.	+	-
May 11 .....	L. A.	++++	++++
May 11 .....	N. B.	++	+
May 11 .....	A. P.	+	±
May 11 .....	F. S.	+	±
May 11 .....	J. B.	±	-
May 11 .....	S. G.	±	-

For those getting results with the diagnostic slide test of maximal specificity but of insufficient sensitivity, it is possible that the addition of more cholesterol to the emulsion may improve the results. As much as 1.25 cc. of cholesterol solution can be present in a satisfactory emulsion made as is the diagnostic test emulsion and totaling 4.4 cc. as does the latter. For each addition of cholesterol solution a correspondingly less amount of saline is added. It is possible that 1.15 cc. of cholesterol solution instead of 1 cc. may be sufficient. Such emulsions give more sensitive results in the diagnostic slide test than does the standard emulsion but their specificity may not be maximal.



A good control emulsion for the diagnostic slide test that gives results of maximal specificity but of a sensitivity somewhat less than that of standard emulsion is one containing 0.8 cc. of cholesterin instead of the routine 1 cc. This control emulsion is made in the same manner as the standard emulsion and may be prepared with half the usual quantities in a 4 x  $\frac{1}{2}$ -inch test tube with a cork stopper according to the following formula: 0.425 cc. distilled water; 0.4 cc. 1 per cent cholesterin solution; 0.06 cc. antigen (not 0.05 cc.); 1.25 cc. physiological salt solution.

Protocol 1 shows the comparatively lesser sensitivity of the control diagnostic emulsion than the standard diagnostic emulsion in cases of syphilis.

#### CONTROL EMULSIONS FOR THE SLIDE TEST FOR THE EXCLUSION OF SYPHILIS WITH HEATED SERUM

Tests for syphilis of standard sensitivity usually give negative results early in syphilis, and for some time before adequate treatment is completed. For this reason such terms as "seronegative" and "seropositive" primary syphilis are employed and the treatment of the disease continued long after the blood tests have remained or become negative. With the development of more sensitive tests for syphilis which detect much smaller quantities of reagin than previously, and with the knowledge of the very prompt penetration of the organisms from the site of entry and of the early reaction in the tissues and blood, it is apparent now that the term "seronegative primary" is unfortunate and really not applicable if a very highly sensitive blood test is employed. Such a test as the standard exclusion slide test, or better still, one of the more sensitive modifications described below detects the disease so shortly after the appearance of the primary lesion (usually within the first week) that the seronegative phase of the disease is negligible and the term hardly justifiable. Furthermore, these tests do not become negative in treated syphilitics until the therapy is about adequate and accordingly they serve as excellent guides and are helpful in keeping the patient under treatment for months after tests of standard sensitivity have become negative. With this newer technic, serological and

clinical cure approximate one another very closely. Such highly sensitive tests are especially valuable for the detection and control of syphilis in the community, for the better control of adequate treatment, and are imperative in the prevention of transfusion syphilis. Since the exclusion slide tests may be performed rapidly and since the comparatively stable emulsions are ready for instant use, they are especially valuable for the latter purpose. (It must be remembered that the spirocheta pallida are most numerous in the blood early in the disease when there is but very little reagin present.) Although the specificity of the exclusion test is not maximal it is high and satisfactory for practical purposes.

The following control emulsions for the exclusion slide test give more sensitive though less specific results than does the standard exclusion test emulsion.

*A. Control exclusion test emulsion containing standard antigen, prepared differently than standard and with half the quantity of ingredients, as follows:*

1. Pipet into a 1 ounce bottle 0.5 cc. of 1 per cent standard cholesterin solution.
2. Pipeting with the left hand, allow 0.425 cc. of distilled water to mix drop by drop with the cholesterol solution, rotating the bottle with the right hand during the mixture and for 10 seconds thereafter on a flat surface.
3. Add 0.06 cc. (not 0.05 cc.) of standard antigen and after 5 to 10 seconds gently rotate the bottle in upright position on a flat surface for 5 seconds.
4. Allow to stand 1 minute and again gently rotate the bottle in upright position on a flat surface for 5 seconds.
5. Add 1.25 cc. physiological saline solution and gently rotate bottle in upright position for five seconds.
6. Gently pour the emulsion into a narrow test tube and place in the water bath at 56°C. for 15 minutes.
7. Transfer gently to 3 x 1 inch (colloidal gold) tube.
8. Centrifuge 5 minutes at the eighth rheostat setting as for the standard exclusion slide test emulsion.
9. Decant the supernatant fluid and take up the sediment in about 1 cc. of saline (it may require a little experimentation to determine the proper amount of saline to add to obtain the optimal number of particles in the emulsion).

*Vigorous shaking and rotation in the preparation and subsequent handling of the emulsion must be avoided.*

Emulsions properly prepared and properly handled have been found satisfactory for use for at least 24 hours after preparation.

It is possible that a control emulsion for the exclusion test prepared with a mixture of about eight parts of standard slide test antigen and about two parts of an antigen containing some adventitious material (prepared as is standard slide test antigen but with 95 per cent ethyl alcohol instead of absolute alcohol), in the same manner as that described above, may be an even more satisfactory emulsion for the exclusion of syphilis in spite of its giving a greater number of false positive reactions.

*B. Control exclusion test emulsion prepared as is the standard exclusion test emulsion but with antigen containing some adventitious material.*

Attempts were made to increase the sensitivity of the exclusion slide test emulsion in a number of ways including the heating of the antigen wax and the antigen solution before its addition to the emulsion and by the addition to standard antigen of various substances, as such or in alcoholic or water solution. Among these were the following: Sodium chloride, sodium oleate, sodium hyposulphite, sodium nitrite, glacial acetic acid, orthophosphoric acid, metaphosphoric acid, oleic acid, hydrogen peroxide, acetone, cholesterin, gum mastic, choline hydrochloride, glycerin, maltose, butter, suet, and olive oil. The results of all these mixtures were unsatisfactory. One method of value in increasing the sensitivity of the exclusion test emulsion, although at a cost of slight additional false positivity, was the use of 0.065 cc. standard antigen and 0.035 cc. of an antigen prepared as for the routine slide tests but with 95 per cent ethyl alcohol instead of with absolute alcohol in place of 0.1 cc. standard antigen. Such a control emulsion for the exclusion test is made in the same manner as is the standard emulsion and may be prepared with half the usual quantities in a 4- x  $\frac{1}{2}$ -inch test tube with a cork stopper. The following formula is suggested: 0.425 cc. distilled water; 0.5 cc. 1 per cent cholesterin solution; 0.06 cc. of antigen (not 0.05 cc.) (a mixture of 7 parts standard antigen, 3 parts antigen made with 95 per cent alcohol); 1.25 cc. physiological salt solution. After the ingredients are mixed, the emulsion is heated at 56°C. in the tube in which it is prepared and all of it is poured into a 3- x 1-inch centrifuge tube and handled thereafter in the routine manner.

Protocol 2 shows the greater sensitivity though lesser specificity of the A and B control exclusion test emulsions as compared to the standard exclusion test emulsion.

# PROTOCOL 2

## *Exclusion slide tests in cases of syphilis emulsions*

DATE	PATIENT	STANDARD METHOD OF PREPARATION		MODIFIED METHOD OF PREPARATION (CHOLESTERIN FIRST, GENTLE ROTATION)	
		1 Standard antigen	2 0.065 cc. of standard antigen; 0.035 antigen prepared with 95% alcohol	3 Standard antigen	4 0.08 cc. of standard antigen; 0.02 cc. antigen prepared with 95% alcohol
1937					
March 30 .....	N. H.	—		+	
March 30 .....	K. M.	±		++	
March 30 .....	J. K.	+		++	
March 30 .....	T. H.	++		++++	
March 30 .....	I. B.	+++		++++	
April 16 .....	P. A.	—		±	±
April 16 .....	T. A.	±		+	+
April 16 .....	J. D.	+		++	+++
April 16 .....	L. H.	++		++++	++++
April 16 .....	M. K.	+++		++++	++++
April 19 .....	J. S.	±		±	±
April 19 .....	W. M.	+		+++	++++
April 19 .....	M. B.	+		++	++
April 19 .....	L. C.	+		+	++
April 19 .....	E. L.	++		+++	++++
April 20 .....	C. S.	—		±	±
April 20 .....	A. B.	±		+	+++
April 20 .....	G. F.	+		+++	+++
April 20 .....	R. R.	+		++	++
April 26 .....	S. P.	±		+	++
April 26 .....	F. V.	++		+++	++++
April 26 .....	W. M.	++		+++	+++
April 26 .....	E. F.	+++		++++	++++
March 15 .....	S. P.	—	±	±	
March 15 .....	P. S.	±	+	++	
March 15 .....	L. C.	±	+	++	
March 15 .....	S. Z.	+	++	++	
April 14 .....	F. E.	±	++	+	+
April 14 .....	A. B.	+	++++	++	++++
April 14 .....	M. B.	+	+++	++	+++
April 14 .....	A. R.	++	++++	++++	++++

Occasional positive or weakly positive results were given in cases with no evidence or questionable evidence of syphilis by exclusion tests with emulsions in the following order: 2, 2 and 4, 3, 1 (most numerous with 2 and 4, least numerous with 1).

#### SUMMARY

Control emulsions for the microscopic slide precipitation tests for the diagnosis and exclusion of syphilis with heated serum are described above. The control emulsion for the diagnostic test gives somewhat less sensitive results than does the standard diagnostic test emulsion and serves as an excellent control of the specificity of that emulsion. The control emulsions for the exclusion test give more sensitive though less specific results and more surely exclude syphilis than does the standard exclusion test emulsion.

I wish to acknowledge the excellent technical assistance of Mrs. Dorothy Lloyd in testing the various emulsions.

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## HEMATOLOGICAL OBSERVATIONS ON BONE MARROW OBTAINED BY STERNAL PUNCTURE\*

PETER VOGEL, LOWELL A. ERF AND NATHAN ROSENTHAL

*From the Medical Department and the Laboratories of the Mt. Sinai Hospital,  
New York, N. Y.*

(Continued)

### *B. Red Blood Cell Disturbances*

1. *Pernicious Anemia.* Seventeen cases of pernicious anemia were studied; ten had received liver treatment, and seven were in their first known relapse. A marked increase in the immature red cells occurred during the period of relapse. As a result of liver treatment, the marrow regained its normal components and it was impossible to recognize its previous activity. The white cell components varied little from the normal during relapse or remission (table 3).

*Case M. M.* (Method 2). A large, well-developed, well-nourished, pale Italian, 54 years of age, gave a negative history except for a poor diet during the past two years. The patient presented classical signs and symptoms of pernicious anemia.

*Peripheral Blood.* Hemoglobin 31 per cent; red blood cells 1,400,000; white blood cells 4,000; platelets 90,000; reticulocytes 1.8 per cent. *Differential:* Non-segmented neutrophils 1 per cent; segmented neutrophils 72 per cent; eosinophils 4 per cent; basophils 1 per cent; lymphocytes 20 per cent; monocytes 2 per cent.

*Marrow.* Hemoglobin 31 per cent; red blood cells 750,000; total nucleated cells 16,200; platelets 130,000; red cell volume 16 per cent; white cell volume 2 per cent; total white blood cells 8,800. *Differential:* Myeloblasts 2.8 per cent; neutrophilic myelocytes 21 per cent; non-segmented neutrophils 34 per cent; segmented neutrophils 24 per cent; eosinophilic myelocytes 1.8 per cent; non-segmented eosinophils 0.2 per cent; segmented eosinophils 4.4 per cent; lymphocytes 10.6 per cent; reticulum cells 0.4 per cent; endothelial cells 1.2 per cent; megakaryocytes 0.4 per cent; total nucleated red cells 7,400; megaloblasts 32 per 100 white cells; erythroblasts 31 per 100 white cells; normoblasts 21 per 100 white cells.

A second puncture after two and one-half weeks of liver therapy, revealed

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the following: megaloblasts 6.2 per 100 white cells; erythroblasts 27 per 100 white cells; normoblasts 21 per 100 white cells.

2. *Sprue*. Five cases were studied; in three, liver therapy was given, and two were untreated cases in their first relapse. The marrow of these patients was similar to those in the pernicious anemia group. Erythropoiesis was marked during relapse; this gradually returned to normal during liver treatment.

*Case M. V.* (Method 2). A poorly-nourished woman, age 49 years, entered the hospital complaining of diarrhea, pallor, weakness and loss of weight. She showed a macrocytic anemia, low blood calcium, high stool fat (36 per cent) and a flat sugar tolerance curve. She improved rapidly on intramuscular liver therapy. The marrow findings were similar to the aforementioned case of pernicious anemia.

3. *Hemolytic Jaundice*. Nine cases were studied—three acute cases, four chronic cases, and two splenectomized cases. The marrow was normoblastic in the acute cases (Lowinger<sup>15</sup>), but practically normal in the chronic types. In one of the splenectomized patients, myelocytes were observed in the peripheral blood before splenectomy, but the marrow showed no myelocytic increase. Following operation, the peripheral blood returned to normal.

*Case H. S.* (Method 2). A well-developed man, 53 years of age, had been complaining of anemia and jaundice for six months. Both the spleen and liver extended one finger below the costal margin. Pernicious anemia was suspected at first, but free hydrochloric acid was present in the stomach and the fragility of the red cells was increased (0.56–0.36). The marrow was normoblastic.

4. *Hemolytic Anemia*. Four cases were studied. All showed marked anemia, and all succumbed. The marrow findings revealed an erythroblastic-normoblastic response, but not as marked as was found in the hemolytic jaundice cases. The white cell differentials were within normal limits.

5. *Sickle-Cell Anemia*. Two cases presented increased erythropoiesis, with many mitotic figures. There was sickling of the marrow red cells and a marked erythroblastic-normoblastic response.

6. *Polycythemia*. Nineteen cases were studied, three cases without treatment; twelve cases treated with x-ray, acetylphenylhydrazine and phlebotomy; two cases of spent polycythemia; and two cases of secondary polycythemia. In the treated, the untreated, and the secondary polycythemias, bone marrow findings were almost identical. The mature neutrophilic cell predominated, suggesting an excessive maturation factor. There was little erythropoiesis. The megakaryocytes were within normal limits; occasionally there was an increase. No marrow could be aspirated in the two cases of spent polycythemia. On biopsy examination, a fibrotic marrow was found in one of the cases.

The sternal marrow aspiration disclosed findings almost similar to those from marrow obtained by surgical biopsy.

7. *Secondary Anemia*. Twelve cases were included in this group, namely

Plummer-Vinson syndrome, nutritional anemia, anemia of pregnancy, splenic anemia, aregenerative anemia, osteosclerotic anemia, idiopathic achlorhydric or hypochromic anemia. The white cell elements were present in normal numbers. Moderate erythropoiesis (Jagic and Klima<sup>14</sup>) was present only in the nutritional anemia cases.

*Plummer-Vinson Syndrome. Case M. L. (Method 2).* A well-nourished, pale woman, aged 48 years, gave a poor dietary history for the past two years. The symptoms of dysphagia developed two weeks previous to admission. Hypochlorhydria was present. The patient recovered following iron medication. The bone marrow showed an erythroblastic, normoblastic response. (There were numerous macro-normoblasts, several of which had many nuclei.)

8. *Chronic Congenital Aregenerative Anemia. Case B. O. (Method 2).* A well-developed and well-nourished, pale, male child, 4 years old, developed anemia shortly after birth. In spite of repeated transfusions at frequent intervals, the anemic condition had not improved. Except for the constant low level of hemoglobin and red cells and eosinophilia, the peripheral blood findings were normal. The marrow revealed a high percentage of hematogones (24 per cent), and an increase in eosinophils. The marrow biopsy differential revealed very similar findings.

9. *Aplastic Anemia.* Three cases were studied. There was hypoplasia of all of the elements in the marrow. There was a "shift to the right" in the myeloid elements, and a relative increase in normal lymphocytes.

#### C. Platelet Disturbances

1. *Thrombocytopenic Purpura Hemorrhagica.* Nine cases were studied: seven chronic cases (three with splenectomy), and two secondary cases. The megakaryocytes were within normal limits. There was no apparent variation in the quality or quantity of cytoplasm of the megakaryocytes. Neither was there an increase in megakaryoblasts. There was no apparent change in the megakaryocytes after splenectomy, even though the peripheral platelets had risen to normal. The marrow differentials were within normal limits. Some of the cases showed a lymphocytosis and some of them had an eosinophilia. The cases of secondary purpura showed similar changes.

#### D. Splenopathies

1. *Gaucher's Disease.* Four cases were studied (table 4). The diagnosis was confirmed in these cases by finding typical Gaucher cells by the sternal puncture (Sokolowsky<sup>29</sup>). This procedure allows an absolute diagnosis to be made. The Gaucher cells ranged as follows, in the four cases: 0.1 per cent; 2.0 per cent; 3.0 per cent and 11.0 per cent of the marrow differential. A differential of a smear taken directly from the marrow, by surgical biopsy in one case, showed practically the same count as was found in the puncture smears.

2. *Hodgkin's Disease.* Five cases, three having had intensive radiotherapy during the year. The marrow differentials revealed a slight "shift to the left,"

and in a few cases an increase in eosinophils and reticulum cells. In one of the cases receiving intensive radiotherapy (200 treatments) nearly all of the myeloid cells showed marked toxic granulation. No Dorothy Reed cells were found in any of these cases.

3. *Splenomegaly*. Fifteen cases: four were considered splenic vein thromboses (one of which was studied before and after splenectomy); three cases had positive Wassermanns; and one case had symptoms of multiple splenic infarcts; the rest were unclassified. In the entire group, the marrow revealed very little or no change from the normal. In a few of these cases there was an increase in eosinophils and eosinophilic myelocytes similar to the Hodgkin's group.

4. *Malaria*. Two cases were studied. One acquired the disease while in Mexico; the other was given the malaria as a therapeutic measure in *tabes dorsalis*. The marrow findings were within normal limits. The parasites were readily found in the red cells, and were apparently more numerous in the marrow red cells than in the peripheral red cells (Ghedini<sup>9</sup>, Seyfarth<sup>28</sup>, Caronia<sup>3</sup>, Osgood<sup>20</sup>). This suggests the value of this procedure in questionable cases of malaria.

*Case L. M.* (Method 1). A 24 year old Spanish sailor developed malaria while in Mexico, two months before admission. His response to therapy was typical. Intracellular parasites 69 per 500 white cells, extracellular parasites 25 per 500 white cells, were found in the bone marrow.

5. *Schistosomiasis*. One case studied. The marrow findings were within normal limits, except for a marked increase in eosinophils and eosinophilic myelocytes.

6. *Undulant Fever*. *Case J. H.* (Method 1). A healthy man, 29 years of age, developed an unexplained fever and splenomegaly which persisted for three weeks. An agglutination test for undulant fever was done and found positive in 1/640 dilution. Vaccine therapy was given and the patient improved. The marrow findings showed a slight "shift to the left" in the myeloid elements.

### *E. Neoplasms*

1. *Multiple Myeloma*. Four cases were studied (Gros<sup>10</sup>). This unusual group was interesting because of the variation of the myeloma-cell types. These variations may represent different stages of plasma cell development, ranging from a true plasma cell type to a more undifferentiated one (table 3) (fig. 1).

The cases were confirmed by biopsy of bone, or at autopsy. All had Bence-Jones protein in the urine, and all had x-ray changes of the bones.

The following case illustrates the rapid change in this disease process:

*Case H. R.* (Method 2). A well-developed man, of 51 years, entered the hospital complaining of severe backache. Bence-Jones protein was found in the urine. The x-ray pictures and a sternal marrow puncture revealed no

TABLE 3

	POLYCYTHEMIA		MYELOGENOUS LEUKEMIA		LYMPHATIC LEUKEMIA* (CHRONIC)	INFECTIOUS MONONUCLEOSIS	AGRAULOCYTOSIS†	GAUCHER'S DISEASE	PERNIOUS ANEMIA AND SPUR		MULTIPLE MYELOMA‡	HEMOLYTIC JAUNDICE	APLASTIC ANEMIA
	Acute	Chronic							Un-treated	Treated			
Number of cases.....→	17	11	9		6	6	3	4	9	13	4	9	3
Myeloblasts.....	3.1	69.2	5.6		1.0	1.1	13.0	2.2	2.2	2.25	3.8	2.5	0.5
Myelocytes agranulocytic.....		22.4	0.8			0.5		— (4.3)**		0.15	— (15.7)††		4.0
Myelocytes.....	15.0	3.6	54.3		7.0	15.0	16.0	34.0	33.7	30.9	6.2	31.2	19.1
Non-segmented neutrophils...	26.0	0.9	18.5		9.0	20.6	1.0	29.0	22.0	26.55	9.5	35.1	16.4
Segmented neutrophils.....	51.0	1.3	10.1		17.0	35.0	0.3	20.6	23.8	33.8	43.7	22.5	32.4
Myelocytes eosinophilic.....	2.2	0.1	0.5		0.2	0.8	0.3	1.7	1.7	0.81	0.4	0.8	0.3
Non-segmented eosinophiles...	0.9	0.07	1.0		0.6	0.1	1.0	1.0	1.2	0.7	0.4	0.5	0.3
Segmented eosinophiles.....	0.5	0.03	1.9		0.6	0.1	0.8	1.0	2.5	1.7	1.6	3.2	0.6
Basophiles.....	0.2	0.05	5.3 (0.3)§		0.1	1.2	0.6	0.1	0.33	0.06		0.2	0.6
Lymphocytes.....	14.0	1.7	2.4		64.0	17.3 (2.6)¶	66.0	3.2	14.3	6.37	18.5	4.6	24.2
Hematogones.....	0.4	0.03	0.3		0.3	5.9	1.0	1.0	0.3	0.23	0.7	2.4	2.1
Reticulum cells.....		0.02	0.02										
Megakaryocytes.....	<0.1	0	0.02		0.2	<0.1	0.2	0.3	0.33	0.15	0.1	0.4	0.1
Megaloblasts.....	3.6	0.03	0.02		0.06	0.3	0.4	2.0	44.1	2.2	0.1	5.6	0.5
Erythroblasts.....	10.8	2.6	8.1		5.9	7.8	34.3	18.8	122.9	18.8	7.7	38.0	10.0
Normoblasts.....	16.8	2.8	14.3		3.5	10.4	22.5	30.6	98.1	47.7	5.2	95.4	14.0

\* Acute lymphatic leukemia—see page 444.

† Leucopenic infectious monocytosis—see page 446.

‡ Carcinoma cells—see page 503.

§ Non-segmented.

¶ Lymphoid cells.

\*\* Gaucher cells.

†† Malignant plasma cells.

positive findings on the first visit. The patient returned in three weeks, and at that time the myeloma cells were present in the sternal marrow in large numbers (20 per cent); in addition, there were definite x-ray changes.

2. *Carcinoma*. A study of twelve cases was made, comprising two cases of carcinoma of the colon; one, of the cervix; five of carcinoma of the lungs; one, of the stomach; one, a Wilms tumor of the kidney; and two cases of carcinoma

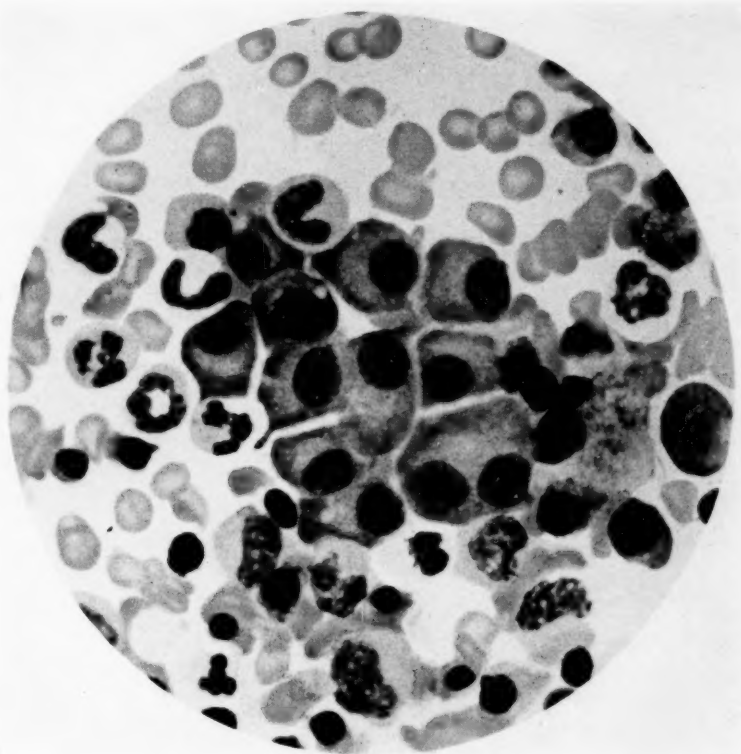


FIG. 1. MULTIPLE MYELOMATOSIS. ( $\times 1000$ .) A NEST OF PLASMA (MYELOMA) CELLS

of the breast with metastases to all bones. A "shift to the right" in the myeloid elements was found. Carcinoma cells were found in the two cases of breast carcinoma with bone metastases. The carcinoma cells were very fragile and remained in groups or clumps (fig. 2).

3. *Sarcoma*. Eight cases of sarcoma were studied. Of these, four cases were lymphosarcoma, one was a case of sarcoma of the uterus; one case was a sarcoma of the stomach, and two were cases of follicular lymphoblastoma.

The marrow findings were essentially normal. No sarcoma or lymphoblastoma cells were found.

*F. Lues*

Four cases of lues were studied: two acquired (secondary stage); one congenital; and one treated. Essentially normal marrow findings were revealed.

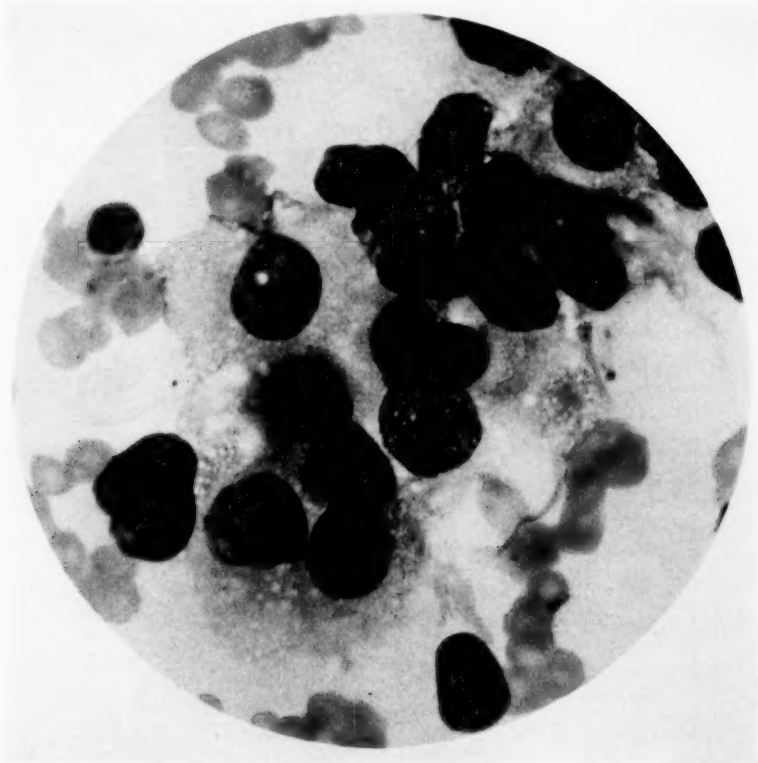


FIG. 2. METASTATIC CARCINOMA. ( $\times 100$ .) A CLUMP OF MALIGNANT CELLS FROM STERNAL MARROW

*G. Miscellaneous Diseases*

1. *Those Showing a "Shift to the Right" in Myelopoiesis of the Marrow.* Five cases were studied: one case each of the following conditions: fatal pulmonary tuberculosis; Felty's syndrome; nephrosis; rheumatic fever; and chronic renal amyloidosis. The last case had a 95 per cent retention of Congo red.

*Case R. S. (Method 2).* A poorly nourished white woman, 34 years of age, with a six year history of ulcerative colitis, entered the hospital with marked



uremic symptoms. The blood urea was 95, and creatinine was 10. The diagnosis of renal amyloidosis was made because of 95 per cent tissue absorption of Congo red.

2. *Those Showing a "Shift to the Left" in Myelopoiesis of the Marrow.* Six cases were studied, one case each of the following conditions: periarteritis nodosa; lupus erythematosus; chronic bronchitis; childhood tuberculosis; lobar pneumonia; and cholangitis. In typhus fever Tuschinsky and Kotlarenko<sup>22</sup> estimate the stage of the disease by the degree of the shift found in the marrow obtained by puncture.

3. *Those Showing Normal Marrow Findings.* Sixteen cases were studied, including acute rheumatic fever, chronic colitis, osteoporosis, polycystic kidney, tuberculosis, pituitary dystrophy, two cases of subacute bacterial endocarditis, osteoarthritis, lupus erythematosus, mycosis fungoides, typhoid fever, obstructive jaundice, bleeding after tooth extraction, tuberculous meningitis, and rectal bleeding.

#### H. Specific Disease Entities

1. *Leprosy (Cutaneous).* The bone marrow findings were essentially normal, except that acid-fast bacilli were revealed in packets with Ziehl-Neelson stain in the marrow smears (Gass and Rishi<sup>3</sup>).

2. *Prematurity.* Two cases were studied. Both were 7 months premature babies, one 12 days old the other 21 days old. The purpose in mentioning these cases (since the marrow findings were normal) is to emphasize the fact that technically the procedure may be used even in a three pound baby. A number 22 gauge spinal puncture needle was used; the marrow was withdrawn without difficulty.

3. *Filariasis.* This case had a history of a hydrocele 2 years previously, in which numerous filaria were found. The urine has since remained milky—which is practically a pathognomonic sign of filariasis, yet no filaria have been observed in the patient's blood. It was thought that the filaria might be present in the marrow, since filaria are photophobic, but none were found.

#### DISCUSSION

Marrow was aspirated from the sternal bone in 246 cases, in some of which repeated punctures were made. In the beginning of this study, actual surgical sternal biopsies were performed for comparison in a number of the cases. Smears made of the puncture material were found, qualitatively, to be nearly identical with those made from biopsy material.

It must be realized that in spite of this similarity the following obvious disadvantages with this procedure must be noted:

First, there is a loss of histological structure of the marrow. The extent and relationship between erythropoietic and myelopoietic foci are absent. Likewise, the distribution of megakaryocytes and fat cells is altered. Second, evaluation of the degree of hyperplasia or hypoplasia is at times difficult. However, the total marrow white count usually indicates the degree of hyperplasia. Third, in less than two per cent of our cases the sternal puncture was unsuccessful. Two of the failures were due to thickening of the sternal plate; and two others had marrow fibrosis, proven later by biopsy. Fourth, there is an admixture of blood when large amounts of marrow fluids are withdrawn. This is largely overcome by withdrawing only 0.1 to 0.2 cc. of marrow fluid, as described in the first method. Arinkin<sup>2</sup> Norden-son<sup>17</sup> and Segerdahl<sup>27</sup> have clearly demonstrated that the first drop of marrow fluid contains the greatest number of marrow cells. The following case illustrates this point:

*Case M. B.* A woman (white) 30 years of age, whose condition was diagnosed clinically as subacute bacterial endocarditis. Simultaneous cultures of marrow fluid and peripheral blood were taken, as suggested by Arinkin<sup>2</sup>. A count on the first drop of marrow fluid revealed 100,000 white cells per cubic millimeter, and 110 megakaryocytes per cubic millimeter. Seventy per cent of the white cells represented immature forms. After 3 cc. of marrow blood was withdrawn for culture, a count taken from the needle showed only 8,000 white cells—all mature forms and no megakaryocytes.

Rib resection was performed in two other patients (one with chronic tuberculosis and the other with a pulmonary neoplasm). The dry rib marrow forcibly expressed by bone forceps was compared with the sternal marrow aspirated at the time of rib resection. Qualitatively the findings were about identical. Quantitatively there were nearly four times as many nucleated cells in the forcibly expressed rib marrow as in that which was aspirated. The former contained approximately one-fourth as many mature red cells. This was due to lack of active circulation in the resected rib.

The advantages of this procedure outweigh the disadvantages. The simplicity of the technic makes it an ideal method for the internist and hematologist. The only equipment necessary is a lumbar puncture needle and a syringe—preferably autoclaved. The procedure is thus adaptable for ward, clinic, home or office use. No special permission is required, as for a biopsy. Re-

peated punctures may be readily and easily done. Nordenson has performed as many as six punctures on one patient the same day. The marrow cells obtained by puncture can be more accurately identified than those studied in marrow sections.

Due to autolysis or decalcification, the puncture procedure allows no cellular changes to occur. It should not be compared with post-mortem or biopsy sections where accurate cytological identification is difficult.

We have been unable to make accurate differential counts in sections, according to the technic of Custer<sup>4</sup>. The sternal puncture is harmless and considerably less painful than a surgical biopsy. No special or surgical training is necessary to insert the needle. There have been no complications or sequelae in over 250 punctures, and no scars result from the procedure. This is of esthetic importance for women and children. The diagnosis is available immediately, and there is no delay, as is necessary when sections have to be made.

The various modifications (Young and Osgood<sup>33</sup>, Reich<sup>21</sup>, Henning<sup>11</sup> and Escudero and Varela<sup>7</sup>) of sternal puncture technic are not superior to Arinkin's<sup>1</sup> original method. Reich<sup>21</sup> uses a specially devised large gauge needle which often requires a mallet for penetration. He then withdraws 10 cc. of marrow fluid and proceeds to centrifuge. The smears are made from the white cell layer. Young and Osgood<sup>33</sup> also withdraw larger amounts and add oxalate to the aspirated marrow which, possibly, accounts for the high percentage of unclassified cells which they obtain.

#### CELLULAR FINDINGS

The sternal marrow is characteristic of general marrow activity. Nordenson<sup>17</sup> aspirated marrow from the vertebra, rib, ileum, tibia, and sternum at the same time, and found the marrow differentials to be similar in all five bones. This was verified in one of our cases in which the sternum, rib, and tibia were punctured simultaneously.

Although the first method (0.1 cc. withdrawn) is preferable, certain findings revealed by the second method may be men-

tioned. In general, the hemoglobin, red cell count and red cell volume in our series were slightly lower than the peripheral values. Similar findings were reported by Tuschinsky and Kotlarenko.<sup>32</sup> In no case was there a marked increase in the marrow red cell count over that of the peripheral red cell, even when the marrow was hyperplastic. The presence of fat globules, which occupy some volume, may explain this difference. The white cell counts and white cell volumes varied according to the hyperplasia of the marrow. The marrow platelet count was nearly always lower than was that of the peripheral platelet count. In a case of thrombocytolytic splenomegaly, the marrow platelet count exceeded the peripheral. In a few cases a platelet layer and fat layer were present but appeared unrelated to any particular group of cases. However, the first method was found more advantageous for this study, since the total nucleated cell count was closely related to the hyperplasia. In addition, the megakaryocytes could be enumerated.

No particular deductions can be made exclusively from the gross aspect of the marrow, since it always appears bloody. It is light red in aplastic and secondary anemias; beet-red in pernicious anemia, and grayish in leukemia.

It is essential—in order to understand the pathological physiology—to correlate the peripheral blood findings with those of the marrow. In most diseases the latter parallel the peripheral findings. However, the sternal marrow frequently retains a more or less normal character, in the presence of well-defined disturbances of the peripheral blood.

Certain inconsistencies are found which may prove important in the physiological studies of the marrow and may materially assist in the prognosis, differentiation, and diagnosis of diseases of the blood and spleen. Thus, peripheral leukopenia may be associated with a hyperplasia or hypoplasia of the marrow, and an anemia, with a decrease or increase of erythropoietic elements.

Further correlation of blood and bone marrow studies may lead to fundamental observations during disease processes. Tempka and Braun<sup>30</sup>, Segerdahl<sup>27</sup> and Nordenson<sup>17</sup>, were able to follow the marrow changes in cases of pernicious anemia undergoing treatment with liver extract. They were able to show the rapid

maturation of red cells from megaloblasts to normoblasts within a short time after treatment with liver extract. Marrow changes may be followed before and after therapeutic procedures such as splenectomy in purpura and hemolytic icterus, and also before and after investigative procedures such as Congo red, India ink, and thorotrast intravenous injections. In one of our patients with 95 per cent tissue absorption of Congo red, determination of the absorption by the marrow was investigated. Twenty cubic centimeters of 1 per cent Congo red were injected intravenously just prior to sternal puncture. None of the dye was found in the marrow in fresh, unstained, wet preparations. Another possibility encountered was the opportunity of studying the phagocytic powers of immature cells (Osgood<sup>20</sup>).

#### MARROW CHANGES OCCURRING IN VARIOUS DISEASES

In certain cases, sternal puncture was of diagnostic importance; in others, of corroborative or confirmatory value; and in still others it offered little or no aid.

*A. Sternal Puncture of Diagnostic Importance.* This applies in Gaucher's disease, myeloma, leishmaniasis (Schreiber<sup>20</sup>), malaria, and in certain cases of leukemia and carcinoma (generalized bone metastases). The importance of puncture in occasional cases is demonstrated by the following example:

*Case S. R.* A man of 52 years, was studied in two general hospitals because of pains in lumbar region and right thigh. The liver and spleen were not palpable; there was a palpable mass on the right thigh fixed to the bone. X-rays revealed rarefaction of the lumbar vertebrae, femur, and pelvic bones. Clinical and roentgenological findings seemed to point to a malignant condition with metastases to bones. The primary lesion could not be determined. By means of sternal puncture the diagnosis of Gaucher's disease was made in this patient without splenomegaly. (Courtesy of Dr. A. A. Berg, Montefiore Hospital, New York City.)

*B. Sternal Puncture of Confirmatory Value.* In the following conditions, puncture of the bone marrow is of confirmatory value; leukemia, leukopenic infectious monocyctosis, agranulocytosis, pernicious anemia, sprue, hemolytic jaundice, polycythemia, and aplastic anemia.

In studying the various leukemias it became evident that bone



marrow puncture was mainly corroborative, and frequently reflected the peripheral blood changes. However, in one case of myeloblastic leukemia the clinical symptoms resembled those of agranulocytosis. The peripheral count revealed no anemia, nor thrombocytopenia, but there was a marked leukopenia (800 white blood cells) with a relative lymphocytosis. Throughout the patient's illness, not more than 10 per cent of immature myeloid cells were found. The bone marrow puncture gave confirmatory evidence of the diagnosis of leukemia. The marrow was hyperplastic with 95 per cent myeloblasts. A month later the patient developed anemia and died within six weeks. (Courtesy of Dr. M. A. Ramirez, of the French Hospital, New York City.)

In chronic myeloid leukemia the bone marrow is predominantly myelocytic. In one particular case (B. U.) the peripheral white blood cells were considerably reduced by x-ray treatment (from 300,000 to 90,000) but the bone marrow remained hyperplastic (400,000 per cubic millimeter; Method 1). It is possible that bone marrow studies in cases treated with x-ray may prove of value. In another case (R. L.) the white blood cells fell to a leukopenic level (300,000 per cubic millimeter to 3,000 per cubic millimeter) following x-ray therapy. The bone marrow simultaneously became depleted since the number of total marrow white cells dropped also (400,000 to 20,000 per cubic millimeter, *Method 1*).

In the previously described case of leukemia with remission the sternal bone marrow punctures reveals the importance of following the marrow changes simultaneously with the blood changes. In this case the marrow showed normal findings during remission, as did the peripheral blood.

In lymphatic leukemia (both leukopenic and leukocythemic) the lymphoblasts varied considerably in number. In the chronic cases few lymphoblasts were found.

There is no doubt that the marrow findings were confirmatory in cases of pernicious anemia and sprue (fig. 3). The megakaryoblasts and erythroblasts were markedly increased. Mitotic figures were frequent. As previously stated, marrow can be studied during liver therapy. Segerdahl<sup>27</sup> believes that a megalo-



blastic marrow will mature to an erythroblastic marrow in twenty-four hours on adequate liver therapy.

In hypochromic or achlorhydric anemia the marrow findings were within normal limits. Occasionally macronormoblasts were found. A normoblastic marrow was present in secondary nu-

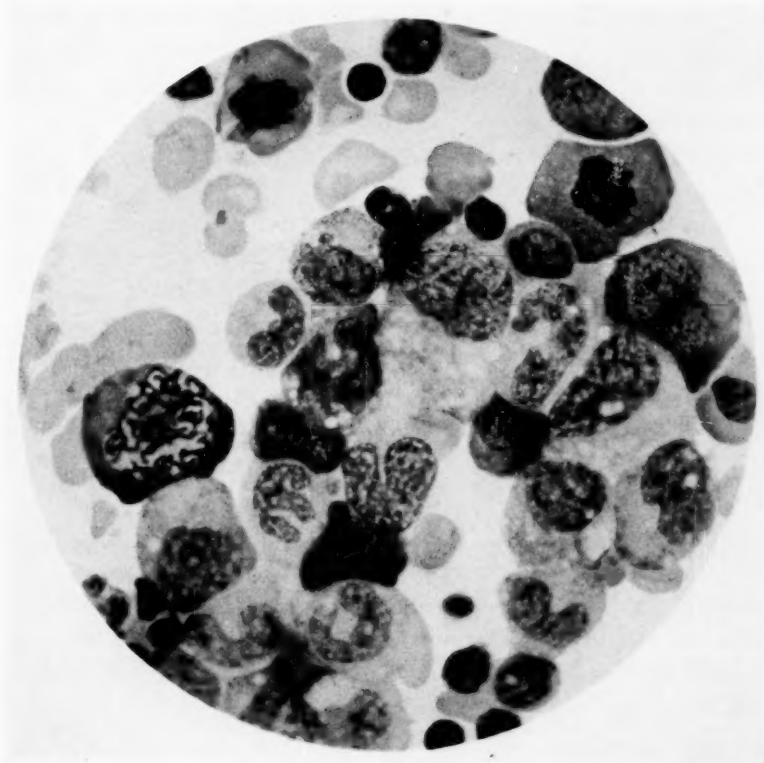


FIG. 3. TROPICAL SPRUE. ( $\times 1000$ .) ILLUSTRATING A MARKED INCREASE OF ERYTHROBLASTS AND MEGALOBLASTS, SOME OF WHICH ARE IN MITOSIS

tritional anemias, as in the cases with loss of blood. Many more cases of hypochromic and secondary anemias must be studied before definite marrow trends can be determined.

The aspirated marrow in aplastic anemia showed a marked decrease in erythropoietic and leukopoietic elements—a true hypoplasia. In one case, it was possible to aspirate only a few

cells and some fatty material. A biopsy made previously showed a marked marrow hypoplasia. This indicated a progressive and continued aplasia.

In hemolytic jaundice the marrow is normoblastic. The puncture allows a rapid differential diagnosis to be made in cases of chronic jaundice.

Marrow puncture smears revealed two interesting findings in polycythemia: (1) the erythropoietic elements were decreased in number, and (2) the predominating cells were mature polymorphonuclear leukocytes.

Agranulocytosis is a most interesting disease from the standpoint of blood and marrow findings. Much information will be obtained from marrow studies during this disease process. Nordenson<sup>17</sup> reports 7 cases, and Segerdahl<sup>27</sup>, 3 cases. Their findings are similar to the 5 cases in this series. In the 3 fatal cases of lymphoid agranulocytosis, there was a depletion of marrow cells with only a small percentage of myelocytes and myeloblasts. No degenerated myeloblasts were present, as Oppikofer<sup>19</sup> reports. However, he used post-mortem sections. The marrow differential in the case of monocytic agranulocytosis (leukopenic infectious monocytosis) with recovery had an increased number of myelocytes and a small percentage of monocytes. Monocytes were found in the marrow in only two of the 246 cases. They were found in the case of benign agranulocytosis and in a case of monocytic leukemia. The total nucleated marrow cells were numerically normal in the benign form of agranulocytosis, but greatly diminished in the fatal lymphoid form. It is possible therefore, to suggest that a normal marrow white cell total count with an increase in myelocytes is a good prognostic sign in agranulocytosis. It appears that a myeloblastic or promyelocytic bone marrow during life is not a common finding. In fact, such a marrow is highly suggestive of leukopenic myeloid leukemia.

In a case of secondary agranulocytosis the primary condition was a splenomegaly associated with a leukopenia of one year duration (the patient had necrosis of the gums, fever, a leukopenia of 800 white blood cells, with 20 per cent neutrophils and 80 per cent lymphocytes). The marrow findings were normal

except for a "shift to the left" (increase of myeloblasts and myelocytes).

*C. Sternal Puncture of Little or No Aid.* In the remaining groups of cases that were studied, the sternal puncture was of little or no aid. Perhaps if repeated punctures had been performed, additional significant findings might have been obtained.

The bone marrow apparently is a very stable organ and has a wide margin of safety.

#### SUMMARY

1. A simple puncture technique (Arinkin) for obtaining sternal marrow is discussed.
2. 246 selected cases were studied and the findings in typical examples are presented.
3. Certain investigative possibilities are suggested.
4. The aspiration of the sternal marrow is of diagnostic, prognostic, and corroborative value in certain diseases.

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CONGENITAL NEUROBLASTOMA OF THE ADRENAL\*  
(SYMPATHOBLASTOMA, SYMPATHOGONIOMA, GANGLIOMA EMBRYO-  
NALE SYMPATHETICUM OR SYMPATHOMA EMBRYONALE)

V. W. BERGSTROM

*From the Department of Pathology of the Binghamton City Hospital and the  
Kilmer Pathological Laboratory, Binghamton, New York*

A review of the literature in 1933 by Scott, Oliver and Oliver<sup>1</sup> who reported four additional cases of neuroblastoma of the adrenal cortex, brought the number to 162. Since then Askin and Geschickter<sup>2</sup> add seventeen, Kellert<sup>3</sup> reported two more to the Pathological Society of Eastern New York, Leinfelder<sup>4</sup> in 1935 adds another, Vander Strateen and Maldague<sup>5</sup> in 1933 four cases, Braithwaite<sup>6</sup> in 1933 one case, Meyer<sup>7</sup> one case, and LeFevre<sup>30</sup> one case, a total of 189. The present case is reported to add one more to the literature and because it presents certain important features not previously recorded. Thus, although a number of cases have been reported as congenital, no one in the literature reviewed indicated that multiple subcutaneous lesions were present at birth, and the skin lesions reported as developing later (Jean Smith<sup>8</sup> and Law, F. W.<sup>9</sup>) were not as extensive as in this case. Furthermore, the age of the patient in this report corresponds to that in which the so-called Pepper<sup>10</sup> type of tumor is generally noted, but here there were no liver metastases, nor was there any exophthalmos as is usually associated with the Hutchinson type<sup>11</sup>.

The patient, a well developed, well nourished, white female infant was born of normal parents whose past history was not remarkable. The pregnancy and delivery were normal and the mother made a prompt recovery. At birth the most striking thing was the presence of multiple nodules or tumors scattered without any pattern or design over the entire surface of the body (see fig. 1), including the scalp, as well as in the soft parts of the mouth. The

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FIG. 1. DISTRIBUTION OF TUMORS ONE WEEK AFTER BIRTH, ALMOST IDENTICAL WITH APPEARANCE ON DAY OF BIRTH



FIG. 2. DISTRIBUTION, INCREASE IN SIZE AND NUMBER OF TUMORS. AUTOPSY, 31 DAYS AFTER BIRTH

tumors varied in size from 0.5 to 4 cm. in diameter. Some projected very slightly above the level of the skin, some were like large nodules on the surface and others could be palpated beneath the surface. They varied in color, some having the appearance of normal skin, others being bluish and an especially large one about the size of a tangerine on the extensor surface of the right forearm was purplish blue suggesting a hemangioma which might rupture at



FIG. 3. SUBCUTANEOUS TUMOR NODULES SEEN IN CUT SECTION

any moment. A bluish halo 3 to 7 mm. wide was present in the skin surrounding the base of almost every tumor. The tumors were firm and fibrous to palpation, showed a tendency to flattening or umbilication, and increased noticeably in size and numbers and became much more hemorrhagic during the last two weeks of life.

An autopsy performed thirty-one days after birth showed the external appearance as already described. The main incision revealed innumerable tumors

in the subcutaneous tissue. In fact, there appeared to be no area free from them in the abdominal wall. Both adrenals were enlarged measuring 6 x 3.5 x 3 cm., being about half again as large as the kidneys to which they were not attached and on which they made no indentation. On cut section the tumors seemed to be multiple in each adrenal, and had a grayish homogeneous appearance with central necrosis and purplish discoloration. A definite capsule surrounded individual tumors and also the conglomerate mass as a whole. In the latter instance the capsule proved on microscopic study to be a thin shell of adrenal cortex. The kidneys both showed tumor nodules not unlike those noted in the skin. Lymph glands found in the perirenal fat were not noticeably enlarged but were hemorrhagic in appearance. In the head, tumors were found in the dura mater, in the periosteum of the frontal bone, and in the choroid plexus and sphenoid bone. No tumors were demonstrated in the orbits but some were present in the subcutaneous tissue in the eyelids and brow. No other tumors could be found in the gross examination.

A histological study of stained sections of liver, lungs, heart, thymus and pancreas revealed no neoplasm. The adrenals showed the presence of large tumor masses expanding the glands and replacing the medulla around which a narrow zone of cortical tissue persists as a capsule. The neoplasm is composed partly of cells of the large lymphoid type with a fairly large zone of clear, pale staining homogeneous cytoplasm in polyhedral or rounded shape depending apparently on pressure. In other places, however, the cytoplasm is so small in amount that it can not be seen. The nuclei are large and vesicular with irregularly distributed chromatin forming clumps on a fine meshwork or distributed as large rods or nodules around the nuclear membrane which is very prominent. Nucleoli are not conspicuous, being found principally in the smaller cells with more homogeneous chromatin in the nuclei. No rosettes can be found and only occasionally a large cell with many nuclei piled up or overlapping each other. Mitoses are fairly numerous being plentiful in some areas and few in others. The cell type is obviously very embryonal in character, being probably very little removed from primitive neural ectoderm. The cells are supported by a reticular network which is very vascular and forms trabeculae, dividing the tumor into many large and small lobules. In general, the fibrous stroma also forms a dense capsule around the tumor sharply separating it from the cortex and often shows hyaline changes. In other places the tumor seems to merge with the fascicular layer of the cortex. In thin areas the cells appear to hang from the fine fibrillae not unlike grapes. Extensive necrosis with hemorrhage is common in the center of the large tumor and there is considerable calcification. Interstitial hemorrhage is marked in the smaller nodules and there is a marked deposition of blood pigment. No medullary tissue can be identified. Both lymph vessels and blood capillaries are increased in number and size, many of the former contain tumor cells in large and small masses and occasionally tumor masses can be found in the blood capillaries.

*Kidneys.* Typical tumor nodules are present in both kidneys. The smaller ones are apparently in the cortico-medullary area and as they grow larger spread into the cortex where they could be seen in the surface, or they would spread into the medulla. The nodules seem to grow partly by infiltration and partly by expansion and many glomeruli and tubules can be found in the midst of some of them. In places there is an abortive attempt at capsulation. Some of the kidney nodules show marked interstitial hemorrhage.

*Spleen.* Numerous large and small nodules are present here and are not different from those in the kidneys and adrenals. Because of the cellularity of the spleen the margins of the nodules are not always definite.

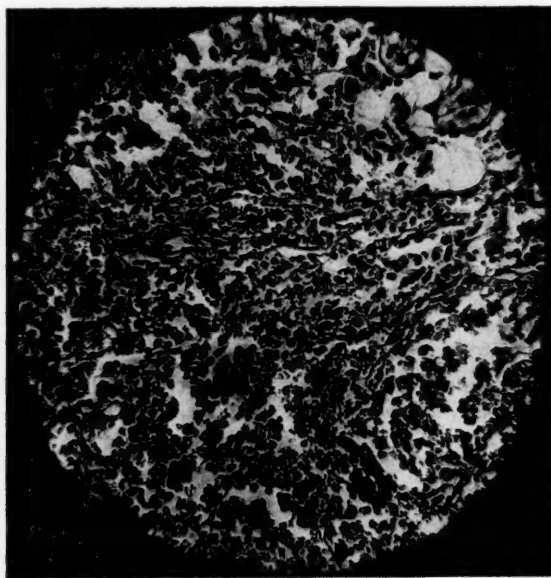


FIG. 4. TUMOR IN KIDNEY. HIGH DRY MAGNIFICATION

*Lymph glands.* These show a lymphosarcomatous appearance with complete loss of normal structure. Low power examination gives the impression that all the cells are small lymphocytes but oil immersion study shows small clumps of reticulum cells or at least cells with a wide zone of cytoplasm, many showing active mitosis. There is very little stroma and the picture is entirely different to that of the tumors described in the adrenal glands, spleen and kidneys.

*Bone.* The cancellous portion of the sphenoid bone shows definite involvement but the thin plate of the frontal bone showed the tumor in the periosteum only.

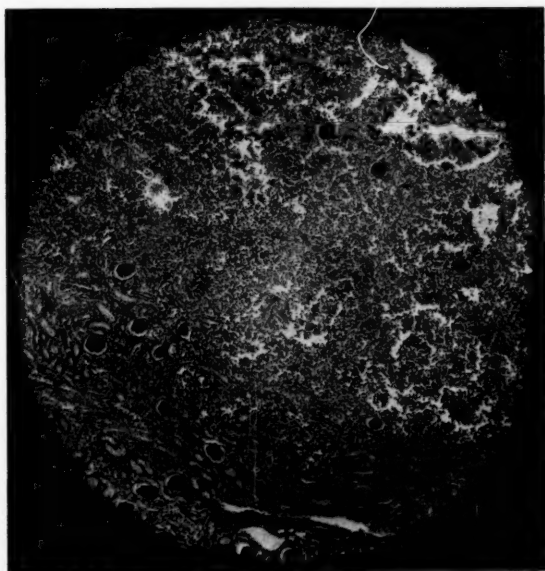


FIG. 5. TUMOR IN KIDNEY SHOWING GLOMERULI. LOW POWER

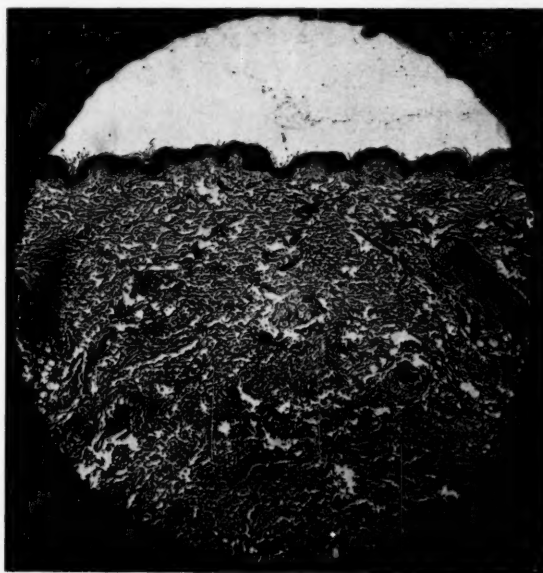


FIG. 6. SECTION THROUGH SKIN NODULE. LOW POWER

*Dura mater.* A single typical tumor nodule microscopically identical with the other tumors was noted.

*Skin.* The lesions in the skin do not differ from the others except that they appear to be more vascular, contain more blood pigment and there is more fibrous stroma. The tumors are in the derma and subcutaneous tissue and a section through the abdominal wall shows multiple tumors reaching from the skin to the muscular fascia. In no instance was a skin nodule found infiltrating the epidermis. There is always a layer of dermis separating them.

#### CONCLUSIONS

The separation of neuroblastomata into Pepper or Hutchinson types is founded on an artificial basis depending on the accidental localization of metastases. Such classifications possess only the merit, inherent through common usage, of indicating the foci of metastases and have the objection of the use of personal names to indicate a common lesion. The presence of tumor cells in both blood and lymph vessels, together with the distribution of the tumors would seem to indicate that the explanations of metastases found in the literature, that is, by way of the lymphatics, Ewing<sup>12</sup>, is not complete. This case supports Pick's<sup>13</sup> contention "that multiple primary tumors may develop spontaneously in the adrenal, liver and elsewhere, a fact of importance in diagnosing metastases." If this is not the case, then the tumors must represent metastases by way of the fetal blood and lymph circulation since the liver and lungs both escaped involvement. The presence of these tumors at birth supports the conclusions of most observers that the lesion is congenital in probably all cases.

#### SUMMARY

A case of congenital neurocytoma with multiple skin nodules is presented. It is believed that this is the first such case to be reported with tumors actually widespread at birth. The distribution of metastases or tumor nodules and the age of the patient do not correspond with the Pepper or Hutchinson types of neuroblastoma.

Two explanations for the occurrence of the skin nodules are given.

This case supports the contention that neuroblastomata are congenital.



I am indebted to Dr. Blinn Buell of the Binghamton City Hospital Staff for the opportunity of studying and reporting this case.

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## THE ETIOLOGY AND PATHOLOGY OF AGRANULOCYTIC ANGINA

### PRESENT-DAY FINDINGS AND HYPOTHESES\*

THOMAS FITZ-HUGH, JR.

*Philadelphia, Pa.*

The fifteen years that have elapsed since Schultz<sup>1</sup> original description of agranulocytic angina may be conveniently divided into three periods. During the first five years the disorder received scant attention, was seldom diagnosed, and, as far as this country is concerned, was probably of rare occurrence. It was generally viewed as a bacterial and toxic destruction of the bone-marrow granulocytes with consequent peripheral granulocytopenia, resulting from overwhelming sepsis with oropharyngeal ulceration as the alleged portal of entry. It was still generally considered, as Schultz described it, a uniformly fatal disease of middle-aged women. A number of observers held it to be nothing more than an unusual type of leucocytic response to a variety of infections<sup>2</sup>. During the second 5-year period, which marks the rapid increase of the disorder in this and other countries it became obvious, first, that not all the victims are elderly women; second; that some recover<sup>3</sup> (at least temporarily) and some exhibit remissions and relapses<sup>4</sup>; third, that granulocytopenia may precede oropharyngeal ulceration and sepsis<sup>5</sup> which are often merely terminal phenomena; fourth, that certain features of the disorder are suggestive of an allergic or sensitivity mechanism<sup>6</sup> with various drugs<sup>7</sup> and bacterial toxins<sup>8</sup> as the chief suspects; and fifth, that treatment with certain metabolites of nucleic acid, adenine sulphate<sup>9</sup>, and pentose nucleotide<sup>10</sup> seem to give the best statistical results (with reduction in mortality to below 50 per cent in some series).

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The third 5-year period begins in 1932 with proof (first presented in the Fall of 1931<sup>11</sup>) that the primary leukopoietic disturbance is not a destruction of leucocytic progenitors, but rather a process comparable to "maturation arrest"<sup>12</sup> with striking discrepancy between the peripheral leukopenia on the one hand, and the relative hyperplasia of the white cell elements of the bone marrow, lymphnodes and spleen on the other. The original view of primary "aplasia" of the myeloid cells of the bone marrow was thus shown to be incorrect, although a terminal aplastic phase (of necrosis) was admitted. Ample confirmation of this finding and hypothesis is now at hand<sup>13, 14, 15, 16, 17</sup>.

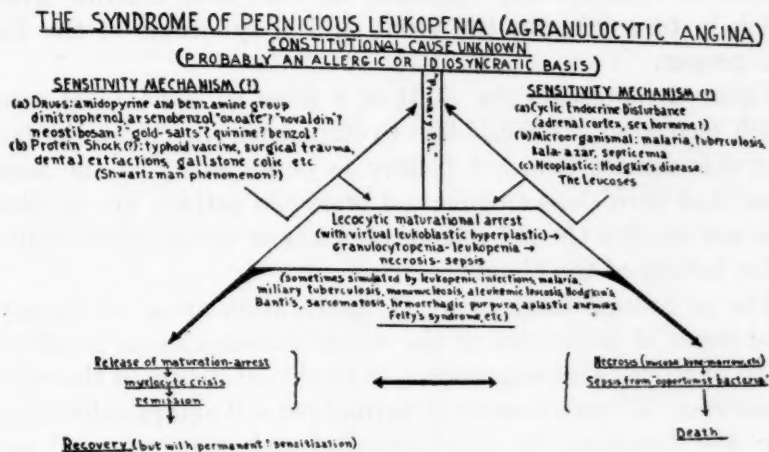


FIG. 1

This last five-year period also signalized the all-important and widely confirmed findings of Madison and Squier<sup>18</sup> concerning the major rôle of amidopyrine idiosyncrasy in the etiology of the disease.

I have attempted to symbolize in figure 1 the chief components of the present day view of the etiology and pathologic physiology of the disorder. It should be stated that many of the supposed causes herein listed (from the data prior to Madison and Squier's paper) are doubtful, because each of them might also involve the ubiquitous amidopyrine whose insidious rôle becomes more

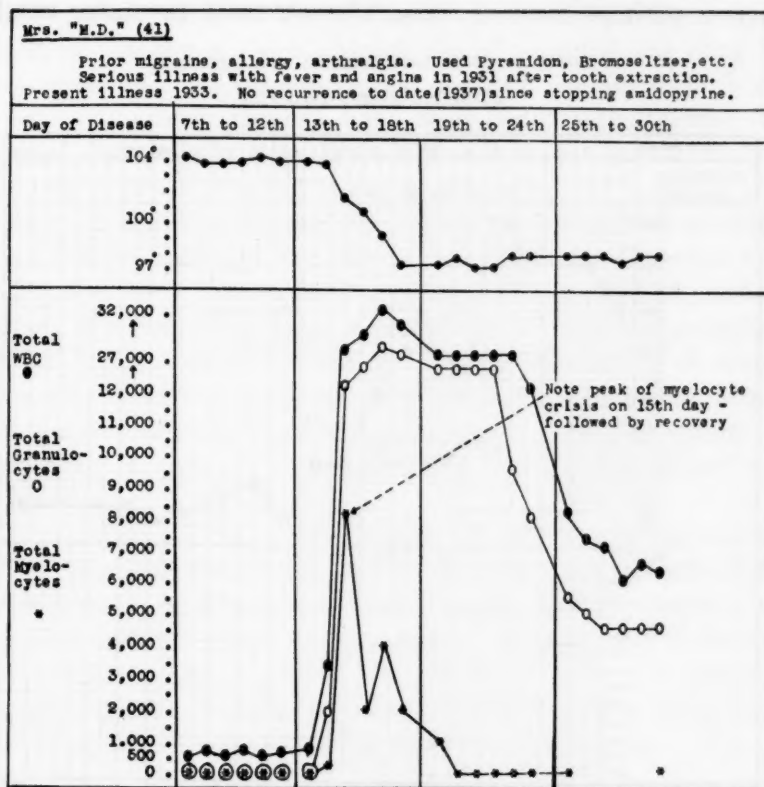
and more obvious as time goes on. Furthermore, there should be an insert somewhere in the schema to indicate the probable significance of the granulocytoclastic crisis phenomenon<sup>19</sup>. In this the sudden changes in the granulocyte count of the peripheral blood (of "sensitized" individuals remaking contact with the drug) occur too rapidly to be mediated by a "maturation arrest" mechanism<sup>20</sup>. It would appear more likely that the "shock mechanism" of this phenomenon is to be explained by sticking of the leucocytes to the capillary endothelium after the fashion which Clarke<sup>21</sup> has demonstrated in his glass-chamber (rabbit-ear) preparations. One might hypothesize that several such granulocytoclastic episodes might precede the final bone marrow arrest which in turn initiates the usual clinical syndrome of the disease proper.

Figure 2 represents the chart of a more or less typical case\* which recovered. I would like to emphasize once more the fact that this disease (which I believe to be an entity in the same sense that pernicious anemia and bronchial asthma are entities) does not involve the red cells or platelets or the so-called coagulation factors of the blood.

The pathologic findings are (1) maturation arrest of the myeloid series of leucocytes at the myeloblast-myelocyte level; (2) arrest or partial non-migration into the blood stream of the other leucocytes; (3) maintenance of normal red cell and platelet structure and function; (4) oropharyngeal and other mucosal and cutaneous ulceration and necrosis with complete or nearly complete absence of polymorphonuclear infiltration; (5) invasion by "opportunistic bacteria" with various types of ensuing sepsis (pneumonia, septicemia, osteomyelitis of the jaw, perforation of the nasal septum, nephritis, intestinal ulceration and necrosis, phlegmonous angina with neighborhood adenitis, endarteritis, acute splenic tumor, acute hepatitis with or without jaundice and finally necrosis of the bone marrow, lymphnodes, adrenals, etc.); (6) recovery initiated by myelocytic hyperplasia of the bone marrow, a myelocyte crisis in the peripheral blood<sup>22</sup> (with extreme

\*The magnitude of the recovery phase of leucocytosis is a little greater than the average peak of about 15,000 which is more commonly seen.

left-shift in the neutrophile formula which here is a happy omen), sometimes a monocytosis as well, and then a return of normal polymorphonuclear and other white cells to the circulation and tissues with ensuing tissue recovery (provided some septic or necrotic process has not already gone beyond repair).



**FIG. 2**

The best statistical results of treatment are those of Henry Jackson, Jr.<sup>16</sup> employing pentose nucleotide (69 recoveries in 103 cases or a mortality of 33 per cent). Favorable results from pentose nucleotide have also been reported in individual cases from various parts of the world. On the other hand, many observers have failed to note any benefit from this therapy, and in my hands it has been of no apparent value. The only explana-

tion I can suggest for most of my own failures on the one hand, and for some of the successes of Jackson and others on the other, is that until Madison and Squier's indictment of amidopyrine appeared I allowed a good many of my patients to continue the use of allonal and other analgesic drugs containing amidopyrine whereas perhaps most of those who obtained favorable results

AGRANULOCYTOSIS (AMIDOPYRINE) with SECONDARY  
HEMOLYTIC STREP. INFECTION.

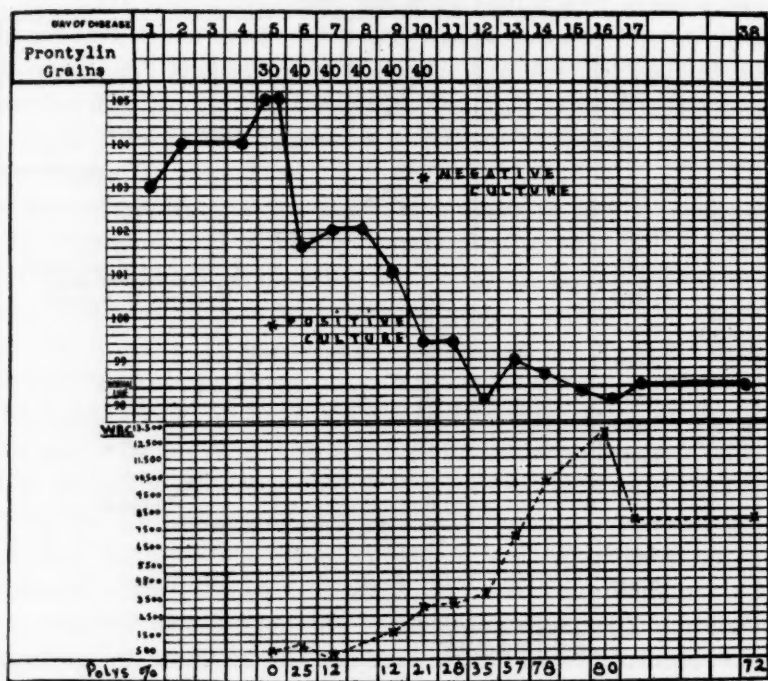


FIG. 3

from pentnucleotide therapy may have (inadvertently or intentionally) omitted this tragically unfortunate symptomatic medication. This may not be the only answer to the problem because Jackson<sup>23</sup> says five of his patients recovered under nucleotide therapy despite continued use of amidopyrine. Looking back on my own statistics, however, it is obvious that my best



results are in the group of patients who stopped ingesting amidopyrine. My present mortality experience to date is 25 dead and 8 recovered (i.e. 75 per cent mortality). Two of the recovered cases and twelve of the fatal cases received pentnucleotide—85 per cent mortality. Seven of my eight recovered cases had taken amidopyrine just prior to the disease and one had taken quinine. Each of the seven "amidopyrine cases" stopped the drug and recovered and has remained well without relapse (one 8 year cure). To match these I have seen eight patients who died despite stopping amidopyrine. This gives an approximately 50 per cent mortality in the group whose treatment included stopping amidopyrine medication, which is a more favorable mortality than I have observed from any other therapy. It would seem reasonable that the earlier the diagnosis is established, and the earlier the offending drug is stopped, the better the outlook.

One other item of therapy relating to etiology is of current interest. Figure 3 presents the chief facts of a recent case of amidopyrine-induced agranulocytic angina, with secondary invasion by streptococcus hemolyticus, successfully treated with prontolin.

Although I would not recommend these benzol-ring sulphonamid compounds promiscuously in cases with already established bone marrow suppression, I think their theoretical danger is outweighed by their value in combating the hemolytic streptococcus. Nonetheless, I predict that sooner or later this group of drugs may be added to the others which in a rare idiosyncrasy rôle may cause the syndrome of agranulocytic angina.

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## CORN WHISKEY AND STRYCHNINE POISONING\*

JACK C. NORRIS

Atlanta, Georgia

During the latter years of prohibition several homicides occurred in which the lethal agent was a combination of strychnine in whiskey. Study of the records in some of these cases demonstrated the fact that whiskey and strychnine in combination was a very quick acting and powerful lethal poison and search of the literature revealed that little if any investigation of the lethal action of the combined substances has been made. No record at all can be found regarding corn whiskey and strychnine poisoning.

In 1934 Gold and Travell<sup>1</sup> reported a series of experiments concerned with the antagonism of ethyl alcohol and strychnine, and in as much as alcohol is the main intoxicating agent in corn whiskey it seemed likely that their studies might be applicable to it. They used rabbits, cats and dogs, and administered the alcohol and strychnine orally, intraperitoneally, and subcutaneously, followed by a lethal dose of strychnine. According to their charts seven fatalities occurred among nineteen animals. In those animals which died the alcohol and strychnine were increased in amounts beyond the minimum lethal dosage. These authors concluded that alcohol in large quantities was an effective antidote to strychnine poisoning in the dog and cat but less effective in rabbits. However, they made one very striking observation which is most significant: *after large doses of alcohol in strychnine poisoning the respiratory mechanism became so sensitive to alcohol that minute quantities intravenously cause cessation of respiration.* They also observed that *after moderate doses of*

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*alcohol and strychnine and more intense poisoning by both drugs convulsions of moderate severity might result in death.*

In August of 1934 Gold and Travell<sup>2</sup> published another report. Cats and dogs were again used in these experiments. In this later report they concluded that a marked antagonism between alcohol and strychnine obtains in only one direction, namely, that alcohol protects the respiratory mechanism against many times the fatal dose of strychnine but strychnine does not protect the respiratory mechanism against more than a single fatal dose of alcohol. Mutual antagonism between alcohol and strychnine does not extend to all the actions of the two drugs in the same degree.

From these reports one may conclude that alcohol is antidotal to strychnine, if administered orally and intravenously to a poisoned animal. However, the animals are not protected against convulsions and the death rate, regardless of what is done, remains approximately 37 per cent. One may also conclude that it is dangerous to administer strychnine and alcohol simultaneously because of the possibility of an increased sensitivity of the respiratory mechanism to the extent that death might occur.

Strychnine and whiskey poisoning have been recognized in several instances in murder cases. On one occasion the murderer admittedly gave his victim strychnine in whiskey as a friendly gesture, encouraging the victim all the while to take larger drinks as a gesture of friendliness, and later followed his drunken victim down various streets gleefully watching him until convulsions and death occurred. In another famous case the victim was directed to whiskey in which strychnine had deliberately been placed. Convulsions occurred ending in death. Not only must this mixture be recognized as a murderous agent of proved effect, but it can give rise to medico-legal problems in other directions. In one instance a middle aged man of good repute and fair health who had been a strychnine taker for a long period, was found dead. It was shown that he had ingested corn liquor to the extent of about one half pint shortly before death. There was also indication that he had simultaneously taken several strychnine tablets. It was believed that he took the two substances

for the purpose of stimulation and rest as there could be shown no reason whatever for suicide. The question arose as to the possibility of accidental death in the case. Was it not possible that the two substances acting in combination in an already sensitized person might produce a lethal effect? There is good scientific reasoning for such a possibility.

Strychnine affects the spinal cord and medullary centers and is a depressant. Toxic doses soon cause tetanus to occur with serious changes in blood pressure, heart action and respiration. Death occurs from respiratory and cardiac failure. On the other hand alcohol or whiskey affects the brain as a whole, promoting at first a stimulation which increases respiration, heart rate and blood pressure. Motion, action and speech are also increased. Caution and inhibitions are decreased under its influence. Finally, absorbed alcohol leads to depression and fatigue and in larger quantities the respiratory center is definitely depressed and the blood pressure falls, sensation and motion become impaired and medullary paralysis follows. The pulse becomes weak and irregular, coma deepens, and finally death results from heart or respiratory paralysis or pulmonary edema. These two drugs are both stimulants and depressants, and both, except for the tetanus in strychnine poisoning, affect the same vital organs. As small a quantity as 0.15 gram ( $\frac{1}{4}$  grain) of strychnine may produce death, while any amount of alcohol from 180 to 450 cc. (6 to 15 ounces) will kill an adult. In considering corn or "moonshine" whiskey as a poison one may reasonably assume it to be a most dangerous substance and frequently a fatal poison. The product of the bootlegger is a substance brewed under the most unsanitary conditions and is difficult to purify. It contains fusel oils, dirt, aldehydes, bacterial proteins and allyl, and often traces of methyl alcohol. It is so potent that only the hardened drinker can tolerate liberal quantities of it and after liberal use leaves its subject in a depressed, sickened, miserable, toxic condition with edema of the brain and a cardiac irregularity. It takes little, if any, imagination to realize the results that might occur if taken together with strychnine by a sensitized person.



## EXPERIMENTAL DATA

The corn whiskey used in the experiments was made by a farmer of good repute and skill and was of fair quality, colorless and clear, sweet and pungent in taste. It burned quickly with a clear blue, smokeless flame and was quite palatable when mixed with sugar and water in a 50 per cent solution. It was mildly toxic to humans and dogs orally. In the human it tended to exhilarate, stimulate, and animate the individual; make him more loving, produce hunger and after eating produce a quiet sound sleep. In the dog similar reactions were observed in that the animal became quite playful, barked frequently and jumped around gleefully, finally settling to a quiet sleep. No nausea, diarrhea or any untoward effect was ever observed except a rapidity of the pulse.

Strychnine, nitrate and sulphate were used in 25 mgm. doses. It was quickly soluble in corn whiskey, and was given orally to different sized, grown dogs.

It was found that corn whiskey diluted with an equal part of water could be given easily by mouth after laying the animal upon its back, holding its legs, and with its head elevated in an assistant's lap. The assistant would then pull out the lateral buccal wall of the mouth and pour the whiskey into this cavity where the fluid would seep between the animal's teeth into his mouth and throat. It was best to sweeten both the strychnine solution and the whiskey. After all fluid was swallowed, water was administered to wash the original fluid into the stomach. The animal was then held in the original position for a period of ten minutes, or until the primary reaction began.

Three plans were followed: (1) whiskey followed by strychnine five minutes later, (2) strychnine followed by whiskey five minutes later, and (3) both substances administered simultaneously. Finally, both were given to an animal to which had been given intoxicating doses of whiskey several times previous to the final poisoning. The amount of whiskey administered was from 30 to 50 cc. In every dog used and given the mixture of corn whiskey and strychnine death occurred in from 30 to 60 minutes.

## CONCLUSIONS

(1) Experimental evidence shows both alcohol and strychnine to be dangerous poisons acting somewhat alike on the brain and spinal cord, and producing death by respiratory and cardiac failure.

(2) Corn whiskey and strychnine in combination are not antidotal, but are lethal poisons.

(3) Corn whiskey and strychnine given simultaneously produce alcoholic stimulation, convulsions and death in animals.



(4) Persons who take strychnine should not take whiskey, and whiskey drinkers should not take strychnine.

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## EDITORIAL

### THE AMERICAN BOARD OF PATHOLOGY

The achievement of The Council on Medical Education and Hospitals of the A. M. A. in standardizing and improving medical education in this country is well recognized, and both the Council and The American College of Surgeons have done a great service in the classification of hospitals. Standardization and improvement in medical education and the classification of hospitals logically preceded the training and acceptance of the medical specialist.

Until comparatively recent times the medical specialist was poorly accepted by the public and only partially recognized by the profession. The term "specialist" was usually applied to those teaching a particular subject or limiting their practices to one field. These specialists gradually formed groups for the advancement of their common interests, and a definite basis on which individuals were to be considered qualified to practice the particular specialty and in turn eligible for membership in the group naturally evolved.

More specific qualifications in a specialty were first crystallized by the ophthalmologists with the organization of their board in 1919. The Boards of Otolaryngology, Obstetrics and Gynecology, and Dermatology and Syphilology were also formed independently, but in 1933 the representatives of these existing boards, along with those of The Association of American Medical Colleges, The American Hospital Association, The Federation of State Medical Boards of the U. S. A., and The National Board of Medical Examiners formed the Advisory Board for Medical Specialties. Further, The Council on Medical Education and Hospitals was authorized by The American Medical Association to formulate standards of administration based upon those of the existing boards and to recognize new boards meeting these standards.

The objectives of an approved special examining board as outlined by The Advisory Board are as follows:

- A. To determine whether candidates have received adequate preparation.
- B. To provide a comprehensive test of the ability and fitness of such candidates.
- C. To certify to the competence of those physicians who have satisfied its requirements.

Using these objectives broadly, special examining boards are now functioning in twelve specialties, as follows: Dermatology and syphilology; internal medicine; obstetrics and gynecology; ophthalmology; orthopedic surgery; otolaryngology; pathology; pediatrics; psychiatry and neurology; radiology; surgery; and urology.

The American Board of Pathology was organized through the joint efforts of The Section on Pathology and Physiology of The American Medical Association and The American Society of Clinical Pathologists. The organization was incorporated in the State of Michigan during the summer of 1936, and is now approved by The Council on Medical Education and Hospitals and The Advisory Board for Medical Specialties. Information regarding the purposes and operation of the Board was first issued in August, 1936. During the intervening year, some four hundred applications have been received, and three hundred and four pathologists have had their qualifications certified at meetings held in Baltimore, Chicago, and Philadelphia.

Conservative estimates indicate that there are about one thousand eligible specialists in pathology at the present time, hence these figures speak for themselves in showing the gratifying response of pathologists to their qualifying board. This wholehearted support of the Board is apparently due to the long felt need for a method of qualification, the success and usefulness of the pioneering specialty boards, and the helpful guidance of The Advisory Board for Medical Specialties. The Board individually and collectively has made every effort to administer the certification work fairly and impartially, adhering closely to the stipulations of the constitution and by-laws drawn up by

the joint committees of The Section on Pathology and Physiology of the A. M. A. and The American Society of Clinical Pathologists, and approved by them, as well as by The Advisory Board for Medical Specialties and The Council on Medical Education and Hospitals of The American Medical Association.

It is the avowed function of this Board to certify every ethical pathologist who voluntarily applies, providing his eligibility and qualifications can be established under the constitution and by-laws mentioned above; therefore, the certification of any individual resolves itself into fact-finding and interpretation. While it is true that there may be differences of opinion regarding some applications on first consideration, the accumulation of further data usually results in a satisfactory decision for all concerned. Eligibility is the most important consideration regarding an applicant, after which comes the method of qualification. For the present, some pathologists may be qualified on their credentials, if they have held professorial rank for five years in an approved medical school or held a senior position in the department of pathology of an approved hospital for ten years. Those unable to qualify under either of these stipulations are qualified by examination. Experience has shown that the examinations are so broad and fundamental in scope that those who are qualified have little difficulty making creditable records. Indeed, some candidates have expressed satisfaction at having been qualified by examination rather than by acceptance of credentials.

Every specialty board was created and functions with one ideal: that of service to the profession, the particular specialty and to the public. The American Board of Pathology cherishes this ideal and hopes that its service will continue to be received, as it has been this past year, in the spirit in which it is offered.

THE TUMOR REGISTRY (SPONSORED BY THE AMERICAN  
SOCIETY OF CLINICAL PATHOLOGISTS)

Every histopathologist is interested in unusual tumors. Every clinical pathologist who aspires to be a competent histopathologist is interested in the study of tumor slides, both the un-

usual and the routine. This has been well demonstrated by the increasingly great popularity of the tumor seminars held in connection with the annual meetings of the American Society of Clinical Pathologists.

The American Society of Clinical Pathologists has this year undertaken the sponsorship of a tumor registry division of the American Registry of Pathology of the National Research Council. This registry is in charge of Lt. Col. J. E. Ash, M.C. Curator of the Army Medical Museum, Washington, D. C., and is one of several registries conducted under the sponsorship of special societies. To this Registry every member of the American Society of Clinical Pathologists should consider it his duty and privilege to submit tumor specimens which will be of scientific or educational value. From this Registry, in turn, will be available sets of slides for study. The loan sets will be distributed through the office of the Society's secretary as soon as details of the plan are complete. The Registry's material is also available at the Army Medical Museum for personal study and, in addition, there will be prepared from this material the slides to be studied and discussed at the annual Tumor Seminar of the Society.

It is obviously impossible for the Registry to be of maximum service until it is supplied with an adequate amount of proper material. It is safe to predict that specimens submitted in accordance with the instruction blanks which Colonel Ash will supply on request, will be studied promptly and thoroughly, and reported upon within a reasonable time. It has been emphasized that this is not a diagnostic service, but one for the preservation and study of instructive tumor material.

Send *NOW* to the Curator, Army Medical Museum, Washington, D. C., for blanks, then forward your interesting or unusual neoplasms to the Registry. If five hundred cases are in the files of the Registry before January, 1938, our sponsorship will be ready to pay dividends.

**DO IT NOW!**

## BOOK REVIEWS

*Clio Medica. Pathology.* By E. B. KRUMBHAAR, M.D., Professor of Pathology, University of Pennsylvania. Cloth, 206 pp., 18 figures, \$2.00. Paul B. Hoeber Inc., New York, N. Y.

Everyone is—or without question should be—familiar with the series of little books edited by Dr. Krumbhaar under the general title of “*Clio Medica*.” To these charming and interesting little thumbnail sketches, as it were, of various phases of medical history is now added the present volume from the facile pen of the Editor.

Dr. Krumbhaar's qualifications, both for the Editorship of the series, and for the preparation of this volume in particular, require no elaboration. Nor is it hardly necessary to say that this present volume is not the least interesting of the series. The medical author, the medical student, the physician and, indeed, even the reading layman will find it of interest. It is impossible to read it without profit, or to open it without interest. Of special interest to the pathologist and physician is the chronological list of “Pathologic Milestones.”

This little volume is a book to be owned, read, and reread.

*Hematological Tables.* By MAX STRUMIA, M.D., Director, Clinical Laboratory, Bryn Mawr Hospital, \$5.00. Published by Bryn Mawr Hospital, Bryn Mawr, Pa.

The purpose of these tables is “to offer students of clinical hematology a simple means of identifying all types of human circulating blood cells.”

The set, which is supplied in a neat and sturdy holder consists of a twenty-three page booklet which contains useful and succinct technical directions, among them a description of the author's universal blood stain as well as modifications of special staining procedures; samples of the hematological reports and graphs used in the Bryn Mawr Hospital; and eight colored plates accompanied by eight explanatory tables.

The author is well known as a hematologist and those who secure this set will not be disappointed in its character or quality. It should prove a useful addition to the laboratory armamentarium, of great value in the teaching of students, and of equal value as a reference when confronted with a puzzling hematological problem.

*Bacteriology of Specific Communicable Diseases: Handbook of Public Health Bacteriology.* Edited by M. S. MARSHALL. Paper, 181 pp., \$1.50. J. W. Stacey Inc., San Francisco, Cal.



In this small handbook, of a size and shape to fit in the pocket and so constructed as to lie flat when opened, are presented the methods used in the various departments of the San Francisco Department of Health under the direction of J. C. Geiger, M.D.

The primary purpose has not been to present a comprehensive manual of technic but to furnish such condensed general information as would enable correlation of the activities of the public health laboratory with the other branches of public health administration.

While confined principally to the methods of the San Francisco Department of Health and mainly to such specimens as properly belong in a health department laboratory, this manual will nevertheless be of interest to laboratory workers in general as a source of much useful information.

*Legal Medicine and Toxicology.* By THOMAS A. GONZALES, M.D., Acting Chief Medical Examiner, City of New York, and Associate Professor of Forensic Medicine, New York University College of Medicine; MORGAN VANCE, M.D., Assistant Medical Examiner, City of New York, Assistant Professor of Forensic Medicine, New York University College of Medicine; and MILTON HELPERN, M.D., Assistant Medical Examiner, City of New York, Assistant Professor of Forensic Medicine, New York University College of Medicine; with a foreword by HARRISON S. MARTLAND, M.D., Chief Medical Examiner, Newark, New Jersey, Professor of Forensic Medicine, New York University College of Medicine. Cloth, 754 pp., 244 illustrations, \$10.00. D. Appleton-Century Co., New York.

The aim of the authors, as stated in their preface, has been to prepare a book "sufficiently complete to use as a source of reference but brief enough to serve the student as an introduction to medicolegal evidence."

Their long and extensive, if not unrivalled, experience is ipso facto evidence of their fitness for such a project. That their aim has been well and satisfactorily achieved even the most cursory scanning of the book suffices to indicate.

While legal medicine and toxicology constitute an important and highly specialized phase of medical practice as such best left to those specially trained in its complexities, nevertheless, certain of its phases may confront the physician in his professional life and others may fall within the province of the clinical pathologist.

Both may be considered fortunate in having such a volume as this to consult as a reference.

Space does not permit a detailed survey of the volume as a whole. Attention may be called, however, to the comprehensive and practical discussions of the lesions encountered at autopsy; to the full descriptions of the various technical procedures applicable to medicolegal investigations; to the discussions of ballistics, and finger printing; to the presentation of the theory and application of isoagglutination tests on human blood; and especially to the section on toxicology including the industrial aspects of toxic substances.

The book throughout is written from the viewpoint of the physician in practice and hence is exceedingly practical.

It may be stated with confidence that this book will achieve almost instant recognition as a standard and, indeed, classic reference text.

The illustrations, derived with but few exceptions from cases handled by the New York Chief Medical Examiner's Office, are of outstanding excellence.

No one will make a mistake in the purchase of this book. It is not a book to be borrowed but a book to be owned.

*A Preface to Nervous Disease.* By STANLEY COBB, M.D., Professor of Neuro-pathology, Harvard Medical School. Cloth, 169 pp., 13 figures, \$2.50. William Wood & Co., Baltimore.

The purpose of this book is to present to the student and physician the facts and correlations necessary to an understanding of the simpler workings of the central nervous system. It has been the author's aim "to mention only those anatomical structures the physiology of which is known, to discuss only physiological processes for which there is at least a fairly well substantiated anatomical correlation, and to describe only the pathology that has fundamental significance."

The result is a volume which, though small, is full of clearly stated information. It can be read with profit by physician and pathologist alike.

*Pathology of The Nervous System.* By J. HENRY BIGGART, M.D., Pathologist to The Scottish Asylums' Board etc. Cloth, 335 pp., 204 illustrations, \$5.25. William Wood & Co., Baltimore.

While primarily intended for the student as an introduction to the pathology of the central nervous system, the pathologist may well add this book to his reference library. He will find it a clear and useful exposition of a complicated subject.

*Symptoms and Signs In Clinical Medicine.* By E. NOBLE CHAMBERLAIN, M.D., Lecturer in Medicine, University of Liverpool. With a chapter on *The Examination of Sick Children*, by NORMAN B. CAPON, M.D., Lecturer on Diseases of Children, University of Liverpool. Cloth, 424 pp., 282 illustrations, 17 in color, \$8.00. William Wood & Co., Baltimore, Md.

The true clinical pathologist is to be found as often by the bedside as in the laboratory. Some degree of familiarity with the problems of clinical medicine—particularly with its diagnostic problems—is therefore essential.

While labeled as an "introduction to medical diagnosis" this book will be found quite comprehensive in its scope. Well planned, well written, and well illustrated it forms a valuable addition to the pathologist's reference library.

*The Laboratory Diagnosis of Syphilis.* By HARRY EAGLE, M.D., Lecturer in Medicine, Johns Hopkins University Medical School, with a *Foreword* by J. EARL MOORE, M.D., Associate in Medicine, Johns Hopkins University.

Cloth, 440 pp., 27 figures and numerous tables, \$5.00. The C. V. Mosby Co., St. Louis, Mo.

Dr. Eagle is well known for his investigations in the field of serology and for his numerous contributions to the literature concerned with the serological study of syphilis.

It is to be expected, therefore, that this book, based upon an extensive practical as well as research experience, should constitute a contribution of notable, even outstanding value.

This expectation is amply fulfilled. The book presents a thorough, comprehensive, and well-balanced survey of the subject in its entirety. Well written in a clear and intelligible style, intelligible not only to the laboratory worker having familiarity with the subject, but also to the physician at large, this book should prove of great value.

To the serologist and laboratory worker it presents in full detail a thorough and complete discussion of the various complement fixation and flocculation methods now accepted as of value. To the physician it furnishes an authoritative and comprehensive survey of their value, their limitations, and their fallacies. All in all, this book without question is destined to take a place among the important and outstanding reference texts on this subject. It may be recommended without reserve.

*The Principles of Bacteriology and Immunity.* By W. W. C. TOPLEY, M.D., Professor of Bacteriology and Immunology, University of London, and G. S. WILSON, M.D., Professor of Bacteriology As Applied To Hygiene, University of London. Cloth, Ed. 2, 1645 pp., 276 figures, William Wood & Co., Baltimore, Md.

This book may be regarded as practically indispensable to the reference library of pathologist, bacteriologist, and physician.

The reputation of the distinguished authors and their many contributions to both bacteriology and immunology alike assures the reader of a well-balanced and authoritative presentation; an expectation more than fulfilled by the book itself.

The present volume includes a full review and discussion of all the many changes and advances which mark the seven years since the first (two-volume) edition. The text shows many changes, and additions and the book may be accepted without reserve as an outstanding text which will serve as a standard reference text for some time to come. Few, indeed, can afford to be without it.

*Shadow On The Land: Syphilis.* By THOMAS PARRAN, M.D., Surgeon-General, United States Public Health Service. Cloth, 309 pp., \$2.50. Reynal and Hitchcock, New York.

In the avalanche of scientific—and not-so-scientific—"literature" which, in recent years has been directed toward the popular education in matters medical, Dr. Parran's book comes as a refreshing contribution. Because of

its freedom from hysteria—real or manufactured for circulation purposes—this book may be recommended as a sane, authoritative presentation which deserves, and will doubtless achieve a wide distribution.

As is now a matter of common knowledge, Dr. Parran has inaugurated a campaign aimed at the eradication of syphilis as a public health problem. This book may be regarded as his "profession of faith," so to speak.

It is well written and, which cannot be too strongly emphasized, devoid of the hysterical ranting unfortunately too common in popular writers on this and similar subjects.

The illustrations, (pictorial statistics) are effective and striking.

Both the medical and general public may read this book with interest and profit.

*Clinical Parasitology.* By CHARLES FRANKLIN CRAIG, M.D., Professor of Tropical Medicine, Tulane University of Louisiana, and ERNEST CARROLL FAUST PH.D., Professor of Parasitology in the Department of Tropical Medicine, Tulane University of Louisiana. Cloth, 733 pp., 243 figures, \$8.50. Lea and Febiger, Philadelphia, Pa.

In view of the reputation of the authors and their many contributions to parasitology in general and clinical parasitology in particular, the appearance of this book by Col. Craig and Dr. Faust will be welcomed.

It is hardly necessary to say that their extensive and practical experience is amply reflected in their book, nor to emphasize the value to pathologist, laboratory worker, and physician alike thus conferred upon this text.

The book is divided into three main sections: I, Protozoa and Protozoan Infections; II, Helminth and Helminthic Infections; III, Arthropods and Human Disease.

In each section the discussion is clear, comprehensive, and authoritative. A Technical Appendix describes in detail the various methods covering the collection and preparation of specimens and their identification.

A list of the important and pertinent references and an author and also a subject index complete the book.

This is a book to be owned. It may be recommended without reserve.

*Manual of Practical Obstetrics.* By O'DONEL BROWNES, Assistant Gynecologist, Sir Patrick Dun's Hospital, Dublin. Cloth, 363 pp., 10 plates, 236 illustrations, \$6.50. William Wood & Co., Baltimore.

This book is addressed to the student and general practitioner who will find it a text reflecting an ample and digested experience. The book is eminently practical in its arrangement and presentation and may well be consulted with profit.

# THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS ROSTER FOR 1937

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BOLIN, ZERA E.....	450 Sutter Street, San Francisco, Calif.
BROWN, CLARK E.....	St. Francis Hospital, Santa Barbara, Cal.
CASE, LUCIUS W.....	131 Lincoln Avenue, Pomona, California
CUMMINS, W. T.....	975 Bush St., San Francisco, Calif.
CUTLER, ORAN I.....	201 San Juan Drive, Loma Linda, Cal.
ELLIOTT, FRANCES P.....	1028-32nd St., San Diego, California
EVANS, NEWTON.....	1100 N. Mission Road, Los Angeles, California
†FOORD, ALVIN G.....	Huntington Memorial Hospital, Pasadena, Calif.
GLENN, ROBERT A.....	Samuel Merritt Hospital, Oakland, Calif.
HAMMACK, ROY W.....	657 S. Westlake Ave., Los Angeles, Calif.
HYLAND, C. M.....	4614 Sunset Blvd., Los Angeles, Calif.
INMAN, J. HEADEN.....	566-567 Haberfelde Bldg., Bakersfield, Cal.
MANER, G. D.....	657 S. Westlake Ave., Los Angeles, Calif.



MICHAEL, PAUL.....	434—30th Street, Oakland, Cal.
MOORE, GERTRUDE.....	2404 Broadway, Oakland, Calif.
PICKARD, RAWSON J.....	805 Watts Bldg., San Diego, Calif.
POTTENGER, J. E.....	Pottenger Sanatorium, Monrovia, Calif.
PRATT, ORLYN B.....	312 N. Boyle Avenue, Los Angeles, California
PULFORD, D. SCHUYLER.....	926 J St., Sacramento, Calif.
RUEDIGER, E. HENRY.....	Mercy Hospital, San Diego, California
SHACKFORD, BARTLETT C.....	701 Professional Building, Long Beach, Calif.
†STOWE, W. PARKER.....	St. Luke's Hospital, San Francisco, Calif.
SUMERLIN, HAROLD S.....	2001 4th Ave., San Diego, California
THOMPSON, HAROLD A.....	907 Medico-Dental Bldg., San Diego, Calif.
ZIEGLER, E. E.....	101 Point Lobos Avenue, San Francisco, Cal.

## COLORADO

CARSON, P. C.....	6119 Mt. View Blvd., Denver, Colo.
CORPER, H. J.....	National Jewish Hospital, Denver, Colo.
DOBOS, EMERIC I.....	St. Joseph's Hospital, Denver, Colorado
DUNLOP, JOSEPHINE N.....	Corwin Hospital, Pueblo, Colo.
FRESHMAN, A. W.....	234 Metropolitan Bldg., Denver, Colorado
HILLKOWITZ, PHILIP.....	234 Metropolitan Bldg., Denver, Colo.
KONWALER, B. E.....	Laboratory, St. Mary Hospital, Pueblo, Colorado
MAYNARD, C. W.....	Pueblo Clinic, 702 N. Main St., Pueblo, Colo.
†MUGRAGE, E. R.....	4200 E. 9th Ave., Denver, Colo.
RYDER, CHAS. T.....	1626 Wood Ave., Colorado Springs, Colo.
STAINES, ETHELYN.....	Burns Bldg., Colorado Springs, Colo.
THORSNESS, E. T.....	Denver General Hospital, Denver, Colo.

## CONNECTICUT

ALLEN, W. M.....	29 Atwood Street, Hartford, Conn.
BEAUCHEMIN, JOSEPH ADELARD.....	Connecticut State Hospital, Middletown, Conn.
BELL, JERRY S.....	Waterbury Hospital, Waterbury, Conn.
FISHER, JESSIE W.....	28 Crescent St., Middletown, Conn.
†HASTINGS, LOUIS P.....	St. Francis Hospital, Hartford, Conn.
KENDALL, R. E.....	30 Lexington Road, Hartford, Conn.
LOUD, N. W.....	New Britain General Hospital, New Britain, Conn.

## DISTRICT OF COLUMBIA

ARONSTEIN, CHARLES G.....	1707 Columbia Road, N.W., Washington, D. C.
BUTLER, C. S.....	Rear Admiral (MC) U. S. N., Naval Medical Center, Washington, D. C.
CAJIGAS, TOMAS.....	1801 Eye St., N.W., Washington, D. C.
**CUMMINGS, HUGH S.....	2219 California Street, N.W., Washington, D. C.
DECOURSEY, ELBERT.....	Army Medical Museum, Washington, D. C.
†HUNTER, OSCAR B.....	1835 Eye St., N.W., Washington, D. C.
KEILTY, ROBERT A.....	1150 Connecticut St., N.W., Washington, D. C.
LEFFLER, HARRISON H.....	900—17th Street, N.W., Washington, D. C.
LINDSAY, J. W.....	1726 Eye Street, N.W., Washington, D. C.
MATZ, PHILIP B.....	Medical Research Subdivision, U. S. Veterans Bureau, Washington, D. C.
**MC COY, G. W.....	U. S. Public Health Service, Washington, D. C.
NEUMAN, LESTER.....	3900 Fulton St., N.W., Washington, D. C.
RICE, E. CLARENCE, JR.....	1726 Eye St., N.W., Washington, D. C.
SELINGER, MAURICE A.....	1726 Eye St., N.W., Washington, D. C.
**STITT, EDWARD R.....	Navy Department, Washington, D. C.
THOMPSON, R. M.....	Army Medical Museum, Washington, D. C.
*VONDERLEHR, R. A.....	U. S. Public Health Service, Washington, D. C.
WHITMORE, E. R.....	2139 Wyoming Avenue, N.W., Washington, D. C.



COLE, R. E.....	P. O. Box 611, Muncie, Ind.
CULBERTSON, CLYDE G.....	3135 College St., Indianapolis, Indiana
GIORDANO, ALFRED S.....	531 N. Main St., South Bend, Ind.
HUNTER, FRANK P.....	617 Life Bldg., Lafayette, Ind.
LYON, M. W.....	122 N. Lafayette Blvd., South Bend, Ind.
MONTGOMERY, LALL G.....	Ball Memorial Hospital, Muncie, Ind.
NICKEL, A. C.....	303 S. Main St., Caylor-Nickel Clinic, Bluffton, Ind.
†RHAMY, B. W.....	347 W. Berry St., Fort Wayne, Ind.
SELSAM, ETTA B.....	511 Merchants Nat'l Bank Bldg., Terre Haute, Ind.
SHIMER, WILL.....	3248 Washington Blvd., Indianapolis, Ind.
THORNTON, H. C.....	Indianapolis City Hosp., Indianapolis, Ind.

## IOWA

HECKER, F. A.....	St. Joseph Hospital, Ottumwa, Iowa
JOHNSON, A. A.....	Council Bluffs Clinic, Council Bluffs, Iowa
KAUMP, DONALD H.....	Iowa Methodist Hospital, Des Moines, Iowa
KESTEL, JOHN L.....	622 Black's Bldg., Waterloo, Iowa
†LAMB, FREDERICK H.....	220 Main Street, Davenport, Iowa
MCNAMARA, F. P.....	Finley Hospital, Dubuque, Iowa
MORGAN, HAROLD W.....	St. Joseph's Mercy Hospital, Mason City, Iowa
STARRY, A. C.....	St. Joseph's Mercy Hospital, Sioux City, Iowa
THATCHER, WILBUR C.....	Physicians Bldg., Fort Dodge, Iowa
WEINGART, JULIUS S.....	Dept. of Path., Iowa Lutheran Hospital, Des Moines, Iowa

## KANSAS

HAMMEL, SETH A.....	114 West Eighth St., Topeka, Kan.
HELLWIG, C. ALEXANDER.....	5651 Van View Pl., St. Francis Hospital, Wichita, Kansas
†LATTIMORE, JOHN L.....	618 Mills Bldg., Topeka, Kan.

## KENTUCKY

BAKER, ALSON.....	719 Pearl St., Berea, Ky.
GORDON, HAROLD.....	Univ. of Louisville School of Medicine, 101 W. Chestnut St., Louisville, Ky.
†MAXWELL, E. S.....	190 N. Upper St., Lexington, Ky.
*SCHERAGO, M.....	University of Kentucky, Lexington, Kentucky
WEETER, HARRY M.....	612 Heyburn Bldg., Louisville, Kentucky

## LOUISIANA

BEVEN, JOHN L.....	1225 Main Street, Baton Rouge, Louisiana
BOWDEN, MARGARET P.....	
HARRISON.....	5665 West End Blvd., New Orleans, Louisiana
BUTLER, WILLIS P.....	P. O. Box 201, Shreveport, La.
**CRAIG, CHARLES F.....	Dept. of Tropical Medicine, Tulane University, New Orleans, La.
†D'AUNOY, RIGNEY.....	1609 Hibernia Bank Bldg., New Orleans, La.
ELLIS, F. G.....	P. O. Box 201, Shreveport, La.
HAUSER, GEORGE H.....	3625 St. Claude Avenue, New Orleans, La.
HEBERT, LOUIS A.....	813 Pujo Street, Lake Charles, La.
LANFORD, JOHN A.....	Touro Infirmary, New Orleans, La.
LAWSON, E. H.....	2700 Napoleon Ave., New Orleans, La.
MAHER, ALDEA.....	1110 Am. Bank Bldg., New Orleans, Louisiana
MATHEWS, WILLIAM R.....	Shreveport Charity Hospital, Shreveport, La.
OGDEN, MICHAEL A.....	2226 Ursuline Ave., New Orleans, La.
PRACHER, JOHN.....	St. Francis Sanitarium, Monroe, La.

- SCHATTENBERG, HERBERT J. . . . . P. O. Station 20, Tulane Univ. Med. School, New Orleans, La.  
 SEEMANN, WILLIAM H. . . . . 1577 Henry Clay Avenue, New Orleans, Louisiana  
 TRIPOLI, CARLO J. . . . . 1212-1214 Union Bldg., New Orleans, Louisiana

## MAINE

- \*LITTLE, CLARENCE C. . . . . P. O. Box 558, Bar Harbor, Maine  
 MORRELL, ARCH H. . . . . 6 Melville, Augusta, Maine  
 †THOMPSON, H. E. . . . . Eastern Maine Gen. Hospital, Bangor, Me.  
 †WARREN, MORTIMER. . . . . Maine General Hospital, 22 Arsenal St., Portland, Me.

## MARYLAND

- COLLENBERG, H. T. . . . . 2 West Read Street, Baltimore, Md.  
 JOHNSON, S. LLOYD. . . . . 1303 Frederick Road, Catonsville, Md.  
 †JUDD, CHAS. C. W. . . . . 8 E. Eager St., Baltimore, Md.  
 MALDEIS, HOWARD J. . . . . 104 W. Madison St., Baltimore, Md.  
 STEWART, C. WILBUR. . . . . Maryland General Hospital, Laboratory, Baltimore, Md.

## MASSACHUSETTS

- BECK, JAMES S. P. . . . . Memorial Hospital, Worcester, Mass.  
 BURNETT, FRANCIS L. . . . . 205 Beacon St., Boston, Mass.  
 CRISCITIELLO, MODESTINO. . . . . 8 Bank Row, Pittsfield, Mass.  
 DALRYMPLE, SIDNEY C. . . . . Newton Hospital, Newton, Mass.  
 FREEMAN, WILLIAM. . . . . P. O. Box 57, Worcester, Massachusetts  
 GLIDDEN, HENRY S. . . . . State Infirmary, Tewksbury, Mass.  
 GOODALE, RAYMOND HAMILTON  
     55 South Lenox, Worcester, Mass.  
 HINTON, WM. A. . . . . 25 Bennett St., Boston, Mass.  
 McCANTS, J. M. . . . . U. S. Naval Hospital, Chelsea, Mass.  
 MORAN, WILLIAM G. . . . . 41 Pondview, Arlington, Mass.  
 RODGER, JAMES Y. . . . . 22 Garden Rd., Lowell, Mass.  
 †SCHADT, GEO. L. . . . . 44 Chestnut St., Springfield, Mass.  
 ULRICH, HELMUTH. . . . . 99 Bay State Road, Boston, Mass.

## MICHIGAN

- AMOLSCH, ARTHUR L. . . . . 3771 W. Philadelphia Ave., Detroit, Michigan  
 BEAVER, DONALD C. . . . . Woman's Hospital, 432 E. Hancock St., Detroit, Mich.  
 BOND, GEORGE L. . . . . 409 Metz Bldg., Grand Rapids, Mich.  
 †BRINES, O. A. . . . . Receiving Hospital, Detroit, Mich.  
 BROSIUS, WILLIAM LEWIS. . . . . 2349 Leslie Avenue, Detroit, Michigan  
 BUGHER, JOHN C. . . . . Univ. of Michigan, Dept. of Pathology, W. Med. Bldg., Ann Arbor, Michigan  
 CLARK, HARRY L. . . . . 634 Maccabees Bldg., Detroit, Mich.  
 COPE, H. E. . . . . 1551-1559 David Whitney Bldg., Detroit, Mich.  
 GAMBLE, W. G., JR. . . . . Physicians Hosp. & Laboratory, Bay City, Mich.  
 GERMAN, WILLIAM M. . . . . Blodgett Hospital, Grand Rapids, Mich.  
 GOULD, SYLVESTER EMANUEL. . . . . Eloise Hospital, Eloise, Michigan  
 GRUHZIT, O. M. . . . . 580 Hampton Road, Grosse Pointe Shores, Grosse Pointe, Mich.  
 HARTMAN, FRANK W. . . . . Henry Ford Hospital, Detroit, Mich.  
 HOWARD, STACY C. . . . . St. Joseph Mercy Hospital, Ann Arbor, Michigan  
 KASPER, JOSEPH ARTHUR. . . . . Herman Kiefer Hospital, Detroit, Mich.  
 LEWIS, W. B. . . . . Battle Creek Sanitarium, Battle Creek, Mich.  
 LOHR, OLIVER W. . . . . 302 S. Jefferson, Saginaw, Mich.  
 MORSE, PLINN F. . . . . Harper Hospital, Detroit, Michigan  
 NEEDLES, ROBERT J. . . . . Henry Ford Hospital, Detroit, Mich.

OWEN, CLARENCE I.....	Grace Hospital, Detroit, Michigan
OWEN, ROBERT G.....	1551-1559 David Whitney Bldg., Detroit, Mich.
PRENTICE, H. R.....	3404 Oakland Drive, Kalamazoo, Michigan
RODERICK, C. E.....	Battle Creek San., Battle Creek, Mich.
ROTH, PAUL.....	Battle Creek Sanitarium, Battle Creek, Mich.
*SHARP, E. A.....	Parke, Davis & Co., Detroit, Mich.
*YAGLE, ELIZABETH M.....	1530 Seward St., Detroit, Michigan

## MINNESOTA

BERDEZ, GEORGE LOUIS.....	St. Mary's Hospital, Duluth, Minn.
BRODERS, A. C.....	Mayo Clinic, Rochester, Minn.
CRAGG, RICHARD.....	Mayo Clinic, Rochester, Minn.
†DRAKE, CHARLES R.....	600 Phys. & Surg. Bldg., Minneapolis, Minn.
HECK, FRANK J.....	Mayo Clinic, Rochester, Minnesota
HENTHORNE, JOHN C.....	102-110 Second Ave., S.W., Rochester, Minn.
IKEDA, KANO.....	Charles T. Miller Hospital, St. Paul, Minn.
KERNOHAN, J. W.....	Mayo Clinic, Rochester, Minn.
KVITRUD, GILBERT.....	1969 Princeton Ave., St. Paul, Minn.
LUFKIN, NATHANIEL H.....	Minneapolis General Hospital, Minneapolis, Minn.
MACCARTY, WM. CARPENTER.....	Mayo Clinic, Rochester, Minn.
MAGATH, THOMAS B.....	Mayo Clinic, Rochester, Minn.
MCDONALD, JOHN R.....	Mayo Clinic, Rochester, Minn.
MERKERT, G. L.....	1245 Medical Arts Bldg., Minneapolis, Minn.
NOBLE, JOHN F.....	Ancker Hospital, 495 Jefferson, St. Paul, Minn.
ROSENOW, EDWARD C.....	Mayo Foundation, Rochester, Minn.
SANFORD, A. H.....	Mayo Clinic, Rochester, Minn.
**SHEARD, CHARLES.....	Mayo Clinic, Rochester, Minn.
STANGL, FRED H.....	101 7th Ave., So., St. Cloud, Minn.
*WATKINS, CHARLES H.....	Mayo Clinic, Rochester, Minn.
WELLBROCK, W. L. A.....	Mayo Clinic, Rochester, Minn.
WELLS, ARTHUR H.....	St. Lukes Hospital, Duluth, Minn.
**WILSON, LOUIS B.....	Mayo Foundation, Rochester, Minn.

## MISSISSIPPI

†LIPPINCOTT, LEON S.....	Vicksburg Sanitarium, Vicksburg, Miss.
WHITE, E. T.....	Leyser Building, Greenville, Miss.

## MISSOURI

HAGEBUSCH, OMER E.....	4500 Olive Street, St. Louis, Mo.
HANAN, ERNEST B.....	Foster Bldg., Bolivar, Mo.
HELWIG, FERDINAND C.....	St. Lukes Hospital, Kansas City, Mo.
IVES, GEORGE.....	3720 Washington Blvd., St. Louis, Mo.
KATZ, SAMUEL DAVID.....	Wall Bldg., 3903 Olive St., St. Louis, Mo.
KERR, RUSSELL W.....	1827 E. 59th Street, Kansas City, Mo.
KLENK, CHAS. L.....	420 Metropolitan Bldg., St. Louis, Mo.
KORITSCHONER, ROBERT.....	4949 Rockhill Road, Kansas City, Mo.
LEDERER, ARTHUR.....	U. S. Vet Hosp., Jefferson Barracks, Mo.
NARR, FREDERICK C.....	Research Hospital, Kansas City, Mo.
NEAL, M. PINSON.....	University of Missouri, Columbia, Mo.
STONE, MURRAY C.....	542 Medical Arts Bldg., Springfield, Mo.
†TRIMBLE, WILLIAM K.....	836 Professional Bldg., Kansas City, Mo.

## MONTANA

†PETERSON, RAYMOND F.....	Murray Clinic, Butte, Montana
WALKER, THOMAS F.....	Medical Arts Bldg., Great Falls, Mont.

## NEBRASKA

BREUER, MILES J.....	925 Stuart Bldg., Lincoln, Neb.
COVEY, GEORGE W.....	805 Sharp Bldg., Lincoln, Neb.

MANNING, ERNEST T.	1407 Medical Arts Bldg., Omaha, Nebr.
MCCURDY, THOMAS	Creighton University, Omaha, Nebraska
MOODY, W. B.	530 Medical Arts Bldg., Omaha, Nebr.
MORAN, CLARENCE S.	Creighton Medical School, Omaha, Neb.
NEELY, J. M.	3026 Puritan Street, Lincoln, Neb.
RUBNITZ, A. S.	Medical Arts Bldg., Omaha, Nebr.
RUSSUM, BENJAMIN C.	2524 N. 55th St., Omaha, Nebr.
†TOLLMAN, JAMES PERRY	42nd & Dewey Ave., Omaha, Nebraska
*WYANDT, MISS HELEN	University of Nebraska, College of Medicine, Omaha, Nebr.

## NEVADA

†PARSONS, LAWRENCE	235 West 6th Street, Reno, Nev.
--------------------	---------------------------------

## NEW JERSEY

ANTOPOL, WILLIAM A.	201 Lyons Ave., Newark, N. J.
BERNHARD, WILLIAM G.	142 Clinton Ave., Newark, N. J.
BOUGHTON, T. H.	Merces Hospital, Trenton, New Jersey
BRAUNSTEIN, WILLIAM P.	831 Boulevard East, Weehawken, New Jersey
BROWN, LEWIS W.	160 Roseville Ave., Newark, New Jersey
CASILLI, ARTHUR RAYMOND	618 Newark Ave., Elizabeth, N. J.
CASSELMAN, A. J.	301 N. 2nd St., Camden, N. J.
*DOMANSKI, THADDEUS J.	145 New York Ave., Jersey City, N. J.
FENDRICK, EDWARD	91 Watson Ave., East Orange, New Jersey
GOLDBERG, SAMUEL A.	169 Gregory Ave., East Orange, Newark, New Jersey
GRAY, JOHN W.	142 Clinton Ave., Newark, N. J.
HALBACH, ROBERT	513 Main Street, Toms River, New Jersey
KAPLAN, HERMAN B.	324-44th St., Union City, N. J.
KILDUFFE, ROBERT A.	Atlantic City Hospital, Atlantic City, N. J.
KIM, GAY B.	St. Joseph's Hospital, Paterson, N. J.
LOWY, O.	190 Clinton Ave., Newark, N. J.
MARTLAND, H. S.	City Hospital, Newark, New Jersey
MINIER, CARL L.	157 Harrison Street, East Orange, N. J.
PONS, CARLOS A.	501 Grand Avenue, Asbury Park, New Jersey
*VON DER LEITH, JOHN F.	921 Bergen Ave., Jersey City, N. J.
†YAGUDA, ASHER	88 Clinton Avenue, Newark, N. J.

## NEW MEXICO

†VAN ATTA, JOHN R.	First Nat'l Bank Bldg., Albuquerque, N. M.
--------------------	--

## NEW YORK

*ABEL, HAROLD A.	225 W. 86th St., New York, N. Y.
ARONSON, WILLIAM	150 E. 182nd St., New York, N. Y.
BAKER, MARGARET A.	Bay Ridge Sanitarium, 437 Ovington Ave., Brook- lyn, N. Y.
BENTZ, CHARLES A.	126 W. Humboldt Pkwy., Buffalo, N. Y.
BERGSTROM, VICTOR W.	21 Park Avenue, Binghamton, New York
BLEYER, LEO F.	117 Lexington Avenue, Elmira, New York
BOETTIGER, CARL	3640 Bowne St., Flushing, N. Y.
BOWER, GEORGE C.	Marcy State Hospital, Marcy, N. Y.
BROOKS, HENRY T.	47 3rd Ave., New York, N. Y.
BROWN, HERBERT R.	215 S. Goodman St., Rochester, N. Y.
BUXBAUM, EDWARD J.	282 Amhurst Ave., Jamaica, N. Y.
CLEMMER, J. J.	136 South Lake Street, Albany, N. Y.
COCHUEU, LINDSLEY F.	205 East 69th St., New York, N. Y.
CONNERY, JOSEPH E.	75 East 55th St., New York, N. Y.
CORNWALL, L. H.	30 East 76th Street, New York, N. Y.



- CURPHEY, THEO. J.....St. John's Hospital, 480 Herkimer St., Brooklyn, N. Y.
- CURTIS, STEPHEN HORACE.....80 1st St., Troy, New York
- DARLINGTON, CHAS. G.....209 East 23rd St., New York, N. Y.
- EGGSTON, ANDREW A.....653 Park Ave., New York, N. Y.
- ELTON, NORMAN W.....Millard Fillmore Hospital, Buffalo, N. Y.
- ERSKINE, EARL B.....Mary Immaculate Hospital, 152-11 89th Ave., Jamaica, N. Y.
- EXTON, WILLIAM G.....135 Central Park W., New York, N. Y.
- FEIN, M. J.....2602 Avenue M, Brooklyn, N. Y.
- GARBER, C. Z.....Roosevelt Hospital, 59th St. & 9th Ave., New York, N. Y.
- GASPAR, ISTVAN ANTAL.....Rochester General Hospital, Rochester, N. Y.
- \*GETTLER, A. O.....400 E. 29th Street, New York, N. Y.
- GILBERT, RUTH.....116 N. Allen St., Albany, N. Y.
- \*GROAT, WILLIAM A.....105 Rugby Rd., Syracuse, N. Y.
- HENDERSON, R. C.....2685 University Avenue, Bronx, New York City, N. Y.
- HILLMAN, OLIVER S.....140 E. 54th St., New York, N. Y.
- JACOBS, WM. F.....408 Richmond Ave., Buffalo, N. Y.
- KELLY, FRANK L.....Director of Lab., U. S. Naval Hospital, Brooklyn, N. Y.
- KELLY, WM. E.....State Hospital, Middletown, N. Y.
- KLEMPERER, PAUL.....370 Central Park West, New York, N. Y.
- LARIMORE, LOUISE DODDRIDGE.....750 Riverside Drive, New York, N. Y.
- LINDSAY, SAMUEL T.....St. Mary's Hospital, Rochester, N. Y.
- LODER, MARGARET M.....United Hosp., Port Chester, New York, N. Y.
- MARTEN, M. EDWARD.....152 Lenox Rd., Brooklyn, N. Y.
- MASLON, MORRIS.....43 Coolidge Ave., Glens Falls, N. Y.
- McCULLOUGH, KENDRICK.....Grasslands Hospital, Valhalla, N. Y.
- MILLER, JOHN K.....Division of Laboratories & Research, Dept. of Health, State of N. Y., Albany, N. Y.
- MOITRIER, W.....1219 Dean St., Brooklyn, N. Y.
- MORRISON, MAURICE.....250 Ocean Parkway, Brooklyn, N. Y.
- MYERS, J. T.....47-3rd Avenue, New York, N. Y.
- PALTAUF, RUDOLF M.....344 W. 72nd St., New York, N. Y.
- PECKHAM, A. L.....17 Adriance Ave., Poughkeepsie, N. Y.
- PRIESTMAN, GORDON.....Kings Park State Hospital, Kings Park, L. I., N. Y.
- \*RABSON, S. MILTON.....166 W. 72nd St., New York, N. Y.
- \*RICHTER, MAURICE N.....630 W. 168th St., New York, N. Y.
- ROSEDALE, RAYMOND SAMUEL.....276 Norwalk Ave., Buffalo, N. Y.
- ROSENTHAL, NATHAN.....51 East 90th St., New York, N. Y.
- †ST. GEORGE, A. V.....19 W. 55th St., New York, N. Y.
- SCHLEIFSTEIN, JOSEPH I.....784 Park Avenue, Albany, New York
- SHAFFER, RUDOLPH J.....163 East 1st St., Corning, New York
- SHIRMER, EMILIE C.....596 St. Marks Ave., Brooklyn, N. Y.
- SILVERMAN, I. JEROME.....1475 Grand Concourse, New York, N. Y.
- SMITH, ESMONDE B.....118-8th Ave., Brooklyn, N. Y.
- SMITH, W. A.....31 Thomas Ave., Batavia, N. Y.
- SONDERN, FREDERIC E.....24 W. 55th St., New York, N. Y.
- STEEN, H. M.....136 South Lake Street, Albany, N. Y.
- STILLMAN, RALPH G.....525 East 68th St., New York Hospital, Room F-512, New York, N. Y.
- STRUTTON, W. R.....Rockland State Hospital, Orangeburg, N. Y.
- TAUB, JACOB.....1807 Seminole Ave., Bronx, N. Y.
- THALHIMER, WILLIAM.....30 Beekman Pl., New York, N. Y.
- †THOMAS, WALTER S.....Clifton Springs San., Clifton Springs, N. Y.
- THRO, WM. C.....1300 York Ave., New York, N. Y.
- VAUGHAN, STUART L.....187 Linwood Ave. Buffalo, New York
- WALKER, THOMAS T.....546 Woolworth Bldg., Watertown, New York

WALL, WILLIAM ARTHUR.....134 Homer Avenue, Cortland, N. Y.  
 WARWICK, MARGARET.....610 Potomac Ave., Buffalo, New York  
 WESCOTT, ADELINE MAY.....70 Dubois St., Newburgh, N. Y.  
 WRIGHT, ARTHUR W.....Union University, Dept. of Pathology, Albany,  
 N. Y.

## NORTH CAROLINA

BARRET, HARVEY P.....P. O. Box 973, Charlotte, North Carolina  
 BULLITT, JAMES B.....Univ. of North Carolina, Chapel Hill, N. C.  
 BYRNES, THOMAS HENDERSON.....Watts Hospital, Durham, North Carolina  
 †TODD, LESTER C.....703 Professional Bldg., Charlotte, N. C.

## NORTH DAKOTA

BRESLICH, PAUL J.....Northwest Clinic, Minot, North Dakota  
 †LARSON, LEONARD W.....Clinic Bldg., Bismarek, N. D.

## OHIO

BROWN, ROBERT NEIL.....Veterans' Administration Facility, c/o Labora-  
 tory, Dayton, Ohio  
 \*DOAN, CHARLES A.....4935 Olentangy Blvd., Columbus, Ohio  
 FALLER, ALBERT.....19 W. 7th St., Cincinnati, Ohio  
 FIDLER, ROSWELL S.....700 North Park Street, Columbus, Ohio  
 GOEHRING, CARL.....312 National Exchange Bank Bldg., Steubenville,  
 Ohio  
 GOLDBLATT, HARRY.....2085 Adelbert Road, Cleveland, Ohio  
 HADEN, RUSSELL L.....2020 E. 93rd St., Cleveland, Ohio  
 HATHAWAY, BURR MARSH.....1365 Delia Ave., Akron, Ohio  
 HERZBERG, MORTIMER.....Jewish Hospital, Cincinnati, Ohio  
 KITZMILLER, KARL V.....3129 Jefferson Ave., Cincinnati, Ohio  
 KLINE, BENJAMIN S.....Mt. Sinai Hospital, Cleveland, Ohio  
 KRAMER, G. B.....Youngstown Hosp. Laboratory, Youngstown,  
 Ohio  
 LEICHLITER, JOHN W.....The Christ Hospital, Cincinnati, Ohio  
 OESTERLIN, E. J.....260 Dover Road, Springfield, Ohio  
 PAYNE, FOY C.....880 Fidelity Bldg., Dayton, Ohio  
 POLING, ROBERT B.....2218 Market Street, Youngstown, Ohio  
 POTTER, F. C.....256 W. Cedar St., Akron, Ohio  
 RAMSEY, THOMAS L.....225 Michigan St., Toledo, Ohio  
 REINHART, HARRY L.....1711 Essex Road, Columbus, Ohio  
 SAYLOR, EDWARD L.....190 North Portage Path, Akron, Ohio  
 †SCHADE, A. H.....320 Michigan St., Toledo, Ohio  
 SHAWKEER, MAX.....Reeves Bldg., Dover, Ohio  
 SHILLING, E. R.....345 E. State St., Columbus, Ohio  
 SIMPSON, WALTER M.....Miami Valley Hospital, Dayton, Ohio  
 SPOHR, CARL.....Ohio State Univ., Columbus, Ohio  
 STEINBERG, BERNHARD.....Toledo Hospital, Toledo, Ohio  
 VON HAAM, EMMERICH.....Ohio State University, Dept. of Pathology,  
 Columbus, Ohio  
 YOUNG, ANNA M.....1800 East 105th Street, Cleveland, Ohio  
 ZBINDEN, THEODORE.....412 Colton Bldg., Toledo, Ohio

## OKLAHOMA

BAILEY, WILLIAM H.....Wesley Hospital, Oklahoma City, Okla.  
 CHAMBERLAIN, ELIZABETH M.....724 Shawnee Ave., Bartlesville, Okla.  
 HUDSON, MARGARET G.....411 Medical Arts Building, Tulsa, Okla.  
 HULL, WAYNE M.....University, Hospital, Oklahoma City, Okla.  
 JETER, H. G.....1200 N. Walker, Oklahoma City, Okla.

KELLER, WILBUR F.	119 N. Brody, Medical Arts Bldg., Oklahoma City, Okla.
MEYERS, W. A.	Camp 870, Cache, Oklahoma
MYERS, R. E.	230 Osler Bldg., Oklahoma City, Okla.
NAUHEIM, HERBERT SALLY	Morningside Hospital, Tulsa, Okla.
†NELSON, I. A.	St. John's Hospital, Tulsa, Okla.
*TURLEY, LOUIS A.	763 Asp Ave., Norman, Okla.

## OREGON

†FOSKETT, H. H.	Medical Arts Bldg., Portland, Ore.
LAWRENCE, HARRIET J.	819 Selling Bldg., Portland, Ore.
MANLOVE, C. H.	5725 S. Stark St., Portland, Ore.
OSGOOD, EDWIN EUGENE	Univ. of Ore. Med. School, Portland, Oregon
ROBERTSON, T. D.	3215 N.E. 15th Avenue, Portland, Ore.

## PENNSYLVANIA

ANDERSON, HORACE B.	U. S. Nat'l Bank Bldg., Johnstown, Pa.
BAKER, M. H.	6045 Bunkerhill St., Pittsburgh, Pa.
BAUER, JOHN T.	Pennsylvania Hospital, 8th and Spruce Streets, Philadelphia, Pa.
BELK, W. P.	Times Medical Building, Ardmore, Pa.
*BOERNER, FRED.	3403 Huey Ave., Drexel Hill, Pa.
BROWN, CLAUDE P.	1930 Chestnut St., Room 603, Philadelphia, Pa.
BRUECKEN, A. J.	St. Francis Hospital, Pittsburgh, Pa.
BRUMBAUGH, A. S.	1312 11th St., Altoona, Pa.
BUCHER, CARL JOSEPH	The Westbury, 15th and Spruce Sts., Philadelphia, Pa.
CAMERO, A. R.	4107 Chester Avenue, Philadelphia, Pa.
CLARK, J. H.	Maple Ave. and Washington Lane, Wyncote, Pa.
COHEN, MORTIMER	5615 Bartlett Street, Pittsburgh, Pa.
CRAWFORD, B. L.	Jefferson Hospital, Philadelphia, Pa.
DALEY, D. F.	214 Chestnut St., Kingston, Pa.
DEWAN, CHARLES H.	604 So. Wilbur Ave., Sayre, Pa.
FETTERMAN, GEORGE H.	Pittsburgh City Hospitals and Homes, Mayview, Pa.
FOWLER, KENNETH	Presbyterian Hospital, Philadelphia, Pa.
FOX, HERBERT	William Pepper Laboratory Hospital of the Univ. of Pa., Philadelphia, Pa.
FUNK, ERWIN DEATERLY	Reading Hospital, Reading, Pa.
GRAY, J. R. T., JR.	408 Market St., Chester, Pa.
HARTMAN, GEO. O.	740 E. State St., Sharon, Pa.
HELMBOLD, THEO. RAYMOND	5215 Celia Pl., Pittsburgh, (24) Pa.
HOLLINGSWORTH, I. PEM., P.	33 So. High St., West Chester, Pa.
†HUNT, HENRY F.	404 Ferry St., Danville, Pa.
JAISHON, PHILIP	10 South Avenue, Media, Pa.
JANJIGIAN, ROBERT R.	1043 Wyoming Ave., Forty Fort, Pa.
JOYCE, F. W.	4001 California Ave., Pittsburgh, Pa.
KASTLIN, GEORGE JACOB	401 Jenkins Bldg., Pittsburgh, Pa.
KEASBEY, LOUISA E.	Lancaster General Hospital, Lancaster, Pa.
KENNEDY, PATRICK JAMES	65 Fairview Avenue, Lansdowne, Pa.
KOLMER, JOHN A.	#1 Montgomery Ave., Bala-Cynwyd, Pa.
KONZELMANN, FRANK W.	3638 N. 21st St., Philadelphia, Pa.
KOTZ, A. L.	Easton, Pa.
*LUEDEERS, CHARLES W.	1930 Chestnut St., Philadelphia, Pa.
LUND, HERBERT	10 N. Mt. Vernon Ave., Uniontown, Pa.
LYNCH, FRANK B., JR.	Germantown Hospital & Disp., Germantown, Philadelphia, Pa.
McCLOSKEY, BERNARD	338 Locust St., Johnstown, Pa.

McCREARY, THOMAS W.	1301 Virginia Ave., Monaca, Pa.
**McFARLAND, JOSEPH	542 W. Hartler St., Mt. Airy, Philadelphia, Pa.
MENLOWE, PATTERSON M.	1231 Evans Ave., McKeesport, Pa.
MERANZE, DAVID R.	7122 Cresheim Road, Germantown, Philadelphia, Pa.
MILSTEAD, LAURENCE C.	Sacred Heart Hospital, Allentown, Pa.
MOYER, RAY P.	1225 Highland Bldg., Pittsburgh, Pa.
PAUL, JOHN D.	3112 N. Broad Street, Philadelphia, Pa.
PUSCH, LEWIS C.	York Hospital, York, Pa.
RATHMELL, THOMAS K.	Norristown, State Hospital, Norristown, Pa.
RAY, HENRY M.	5040 Jenkins Arcade, Pittsburgh, Pa.
†REIMANN, STANLEY P.	Lankenau Hospital, Philadelphia, Pa.
REINERS, CHARLES ROBT.	741 Washington St., Huntingdon, Pa.
RICHARDSON, RUSSELL	320 So. 16th St., Philadelphia, Pa.
ROTH, JOSEPH F.	149 Dana Street, Wilkes Barre, Pa.
ROTHROCK, H. A., JR.	821 N. Bishopthorpe, Bethlehem, Pa.
RUBENSTONE, A. I.	2006 Spruce St., Philadelphia, Pa.
SANDBLAD, A. G.	1701 Union St., McKeesport, Pa.
SAPPINGTON, S. W.	P. O. Box 81, Bryn Mawr, Pa.
SICKEL, GEORGE B.	525 Welsh St., Chester, Pa.
SIMPSON, JOHN C.	920 Swede St., Norristown, Pa.
SMITH, LAWRENCE W.	Temple School of Medicine, Broad Street at Ontario, Philadelphia, Pa.
SOLOFF, LOUIS A.	611 Rising Sun Avenue, Philadelphia, Pa.
SPAETH, WILLIAM L. C.	5000 Jackson St., Frankford, Philadelphia, Pa.
STEWART, HENRY	230 Baltimore St., Gettysburg, Pa.
STRUMIA, MAX M.	Bryn Mawr Hospital, Bryn Mawr, Pa.
TAYLOR, FRANCES D.	State Hospital, Philipsburg, Pa.
VAN HORN, HERMAN H.	2339 North 4th Street, Harrisburg, Pa.
WENNER, JOHN J.	941 Hamilton Street, Allentown, Pa.
WENNER, THOMAS J.	150 S. Washington St., Wilkes Barre, Pa.
WHITE, C. Y.	6611 N. 10th St., Philadelphia, Pa.
WILLETTS, ERNEST W.	Professional Bldg., Pittsburgh, Pa.
WURTZ, JOHN G.	Homeopathic Hospital, 5230 Center Ave., Pittsburgh, Pa.
YARDUMIAN, KRIKOR YEGHIA	821 N. Beatty Street, Pittsburgh, Pa.
ZILLESEN, FREDERICK O.	Easton Hospital, Easton, Pa.

## SOUTH CAROLINA

†JOHNSON, F. B.	Med. Col. of So. Carolina, Charleston, S. C.
LYNCH, KENNETH M.	Medical College of S. C., Charleston S. C.
MOSTELLER, RALPH	447 Kennedy St., Spartanburg, S. C.
LOWDEN, H. H.	2020 Hampton Ave., Columbia, S. C.
RIGBY, HALLIE CLARK	618 Glendale Ave., Spartanburg, S. C.
TOWNSEND, ELEANOR W.	120 Tradd Street, Charleston, S. C.

## TENNESSEE

*HAMILTON, JOSEPH F.	1283 Vinton Avenue, Memphis, Tenn.
LEAKE, N. E.	899 Madison Ave., Memphis, Tenn.
McINTOSH, JOHN A.	1933 Vinton Avenue, Memphis, Tenn.
†MOSS, THOMAS CHESTER	Methodist Hospital, Memphis, Tenn.
SCHMITTOU, L. V.	1122 Exchange Bldg., Memphis, Tenn.
SPITZ, HERMAN	325 Lambuth Bldg., Nashville, Tenn.

## TEXAS

BELL, MARVIN D.	1109 Medical Arts Bldg., Dallas, Tex.
BLACK, J. H.	1405 Medical Arts Bldg., Dallas, Texas
BODANSKY, MEYER	John Sealy Hospital, Galveston, Tex.
BOHLS, S. W.	410 E. 5th St., Austin, Texas

†BRADEN, ALBERT H.....	St. Joseph's Infirmary, Houston, Texas
CALDWELL, GEORGE T.....	Baylor Medical College, Dallas, Texas
GOFORTH, JOHN L.....	3121 Bryan Street, Dallas, Texas
HULSEY, S. H.....	600 W. 10th St., Fort Worth, Texas
JACKSON, J. WARREN.....	913 Norwood Bldg., Austin, Texas
KEILLER, VIOLET HANNAH.....	4218 Austin, Houston, Texas
KEMP, HARDY A.....	Baylor University, Dallas, Texas
LEWIS, SEABORN J.....	507 Goodhue Bldg., Beaumont, Texas
MARR, WILLIAM L.....	610-11 U. S. Nat'l Bank Bldg., Galveston, Tex.
MOORE, JOHN M.....	Medical Arts Bldg., San Antonio, Texas
OWEN, MAY.....	Medical Arts Bldg., Fort Worth, Texas
POWELL, W. N.....	Scott and White Clinic, Temple, Tex.
ROBINSON, J. E.....	Kings Daughters Hospital, Temple, Texas
STOUT, B. F.....	730 Medical Arts Bldg., San Antonio, Texas
STOUT, SIDNEY E.....	1028 Fifth Avenue, Fort Worth, Texas
TERRELL, T. C.....	Medical Arts Bldg., Fort Worth, Texas
TODD, D. A.....	1502 Nix Professional Bldg., San Antonio, Tex.
TURNER, GEORGE.....	913 First Nat'l Bank Bldg., El Paso, Texas
VENABLE, DOUGLAS R.....	2010 Garfield St., Wichita Falls, Tex.
WILLIFORD, HERMAN B.....	927 San Jacinto Bldg., Beaumont, Texas

## UTAH

OGLIVIE, ORIN A.....	916 Military Drive, Salt Lake City, Utah
----------------------	--

## VERMONT

†BUTTLES, E. H.....	457 So. Willard St., Burlington, Vt.
---------------------	--------------------------------------

## VIRGINIA

†BECK, REGENA COOK.....	Stuart Circle Hospital, Richmond, Va.
BRAY, W. E.....	University of Virginia, Charlottesville, Va.
BUDD, SAMUEL W.....	McGuire Clinic, Richmond, Virginia
DARDINSKI, V.....	309 Marion Street, Clarendon, Va.
GRAVES, KENNETH D.....	Medical Arts Bldg., Roanoke, Va.
*MARTIN, WALTER B.....	339 Boush St., Norfolk, Va.
ROCHE, MARY E.....	St. Vincent's Hospital, Norfolk, Va.
SHAW, FREDERICK W.....	2417 Rosewood Avenue, Richmond, Va.
VAUGHAN, WARREN T.....	808 Professional Bldg., Richmond, Va.

## WASHINGTON

BALLE, ALFRED L.....	Providence Hospital, Seattle, Washington
CEFALU, VICTOR.....	1001 Cobb Bldg., Seattle, Wash.
EDGAR, JAMES D.....	1115 Overbluff Rd., Spokane, Washington
JENSEN, CLYDE R.....	1114 Boylston, Seattle, Wash.
MAGNUSSON, G. A.....	1420 Medical & Dental Bldg., Seattle, Wash.
MCCOLL, CHARLES R.....	St. Joseph Hospital, Tacoma, Wash.
NICKSON, D. H.....	4405-55th Street, N.E., Seattle, Wash.
PATTON, FRANK R.....	Paulsen Medical and Dental Bldg., Spokane, Wash.
PATTON, M. M.....	264 Paulsen Bldg., Spokane, Wash.
SHIREY, RALPH W.....	2703 W. Yakima Ave., Yakima, Washington
†STIER, ROBT. F. E.....	478 Medical and Dental Bldg., Spokane, Wash.
TERRY, B. T.....	Tacoma General Hospital, Tacoma, Washington
WEST, P. C.....	Northern State Hospital, Sedro Woolley, Wash.

## WEST VIRGINIA

- CHERRY, S. L.....315 S. Chestnut St., Clarksburg, W. Va.  
 †HODGES, F. C.....800 First Nat'l Bank, Huntington, W. Va.  
 MATTHEWS, A. R. K.....City Laboratory, 717½ Ann St., Parkersburg,  
 W. Va.  
 SHEPPE, WM. M.....Wheeling Clinic, Wheeling, W. Va.  
 SINCLAIR, MARSHALL W.....Bluefield Sanitarium, Bluefield, W. Va.

## WISCONSIN

- ALLEBACH, H. K. B.....Milwaukee Hospital, Milwaukee, Wis.  
 BARTA, E. F.....425 E. Wisconsin Ave., Milwaukee, Wis.  
 DICKELMANN, LORIN ELMER...2212 Doty Street, Oshkosh, Wis.  
 ENZER, NORBERT.....Mt. Sinai Hospital, Milwaukee, Wis.  
 FERNAN-NUNEZ, MARCOS.....561 North 15th St., Milwaukee, Wisconsin  
 FORD, JOHN L.....St. Vincents Hospital, Green Bay, Wis.  
 GRILL, J. C.....School of Medicine, Marquette University, Mil-  
 waukee, Wis.  
 HEISE, H. A.....Columbia Hospital, Milwaukee, Wisconsin  
 PESSIN, SAMUEL B.....720 S. Brooks St., Madison, Wis.  
 SCULLARD, GARNER.....Sacred Heart Hospital, Eau Claire, Wis.  
 SEELMAN, JOHN J.....79 E. Wisconsin Ave., Milwaukee, Wis.  
 STOVALL, W. D.....Service Memorial Institute Bldg., Madison, Wis.  
 †THARINGER, E. L.....23 W. Wisconsin Ave., Milwaukee, Wis.  
 VAN HECKE, LEANDER J.....Milwaukee County Hospital, Wauwatosa, Wis.

## WYOMING

- †ZUCKERMAN, SAMUEL S.....1606 Capital Avenue, Cheyenne, Wyoming

## ALPHABETIC LIST

- \*ABEL, HAROLD A...New York, N. Y.  
 \*\*ACHARD, CHAS.....Paris, France  
 ADAMKIEWICZ, L. L....San Diego, Cal.  
 ALLEBACH, H. K. B...Milwaukee, Wis.  
 ALLEN, W. M.....Hartford, Conn.  
 AMOLSCH, A. L.....Detroit, Mich.  
 ANDERSON, HORACE B.....Johnstown, Pa.  
 ANDREWS, V. L.....Glendale, Calif.  
 ANTOPOL, WILLIAM A...Newark, N. J.  
 ARKIN, AARON.....Chicago, Ill.  
 ARONSON, WILLIAM...New York, N. Y.  
 APPELBACH, CARL W....Chicago, Ill.  
 ARONSTEIN, C. G....Washington, D. C.  
 ASSELSTINE, STANLEY M.....Windsor, Ontario, Can.  
 †AYERS, A. J.....Atlanta, Ga.  
 BAILEY, WM. H....Oklahoma City, Okla.  
 BAKER, ALSON.....Berea, Ky.  
 BAKER, MARGARET A...Brooklyn, N. Y.  
 BAKER, M. H.....Pittsburgh, Pa.  
 BALL, HOWARD A....San Diego, Calif.  
 BALLE, ALFRED L....Seattle, Wash.  
 BANKS, H. McM....Indianapolis, Ind.  
 BARRET, HARVEY P. Charlotte, N. C.  
 BARTA, E. F.....Milwaukee, Wis.  
 BATES, LEWIS B....Ancon, Canal Zone  
 BAUER, J. A.....Hamilton, Canada  
 BAUER, JOHN T....Philadelphia, Pa.  
 BEAUCHEMIN, JOSEPH A.....Middletown, Conn.  
 BEAVER, DONALD C....Detroit, Mich.  
 BECK, JAMES S. P....Worcester, Mass.  
 BECK, REGENA COOK...Richmond, Va.  
 BELK, W. P.....Ardmore, Pa.  
 BELL, JERRY S....Waterbury, Conn.  
 BELL, MARVIN D....Dallas, Texas  
 BENTZ, CHARLES A....Buffalo, N. Y.  
 BERDEZ, GEORGE L....Duluth, Minn.  
 BERGSTROM, VICTOR W.....Binghamton, N. Y.  
 BERNHARD, WM. G....Newark, N. J.  
 BETTIN, M. E....Los Angeles, Cal.  
 BEVEN, JOHN L....Baton Rouge, La.  
 BISHOP, EVERETT L....Atlanta, Ga.  
 BLACK, J. H.....Dallas, Texas  
 BLEYER, LEO F....Elmira, N. Y.  
 BLUM, LEON L....Terre Haute, Ind.  
 BODANSKY, MEYER...Galveston, Tex.  
 \*BOERNER, FRED....Drexel Hill, Pa.  
 BOETTIGER, CARL....Flushing, N. Y.  
 BOGEN, EMIL....Olive View, Calif.  
 BOHLS, S. W....Austin, Texas  
 BOLIN, ZERA E....San Francisco, Calif.  
 BOND, GEO. L....Grand Rapids, Mich.  
 BOUGHTON, T. HARRIS..Trenton, N. J.



- BOWDEN, MARGARET P. H. New Orleans, La.  
 BOWER, GEO. C. Marcy, N. Y.  
 †BRADEN, ALBERT H. Houston, Texas  
 BRAUNSTEIN, WILLIAM P. Weehawken, N. J.  
 †BRAY, W. E. Charlottesville, Va.  
 BRESLICH, PAUL J. Minot, N. Dakota  
 BREUER, MILES J. Lincoln, Neb.  
 †BRINES, O. A. Detroit, Mich.  
 BRODERS, A. C. Rochester, Minn.  
 BROOKS, HENRY T. New York, N. Y.  
 BROSIUS, WILLIAM L. Detroit, Mich.  
 BROWN, CLARK E. Santa Barbara, Cal.  
 BROWN, CLAUDE P. Philadelphia, Pa.  
 BROWN, HERBERT R. Rochester, N. Y.  
 BROWN, LEWIS W. Newark, N. J.  
 BROWN, ROBERT NEIL. Dayton, Ohio  
 BRUECKEN, A. J. Pittsburgh, Pa.  
 BRUMBAUGH, A. S. Altoona, Pa.  
 \*\*BRUMPT, E. Paris, France  
 BUCHER, CARL J. Philadelphia, Pa.  
 BUDD, SAMUEL W. Richmond, Va.  
 BUGHER, JOHN C. Ann Arbor, Mich.  
 BULLITT, JAMES B. Chapel Hill, N. C.  
 BURNETT, FRANCIS L. Boston, Mass.  
 BUTLER, C. S. Washington, D. C.  
 BUTLER, WILLIS P. Shreveport, La.  
 †BUTTLES, E. H. Burlington, Vt.  
 BUXBAUM, EDWARD J. Jamaica, N. Y.  
 BYRNES, THOMAS H. Durham, N. C.  
 CAJIGAS, TOMAS. Washington, D. C.  
 CALDWELL, GEORGE T. Dallas, Texas  
 CAMERO, A. R. Philadelphia, Pa.  
 CARSON, P. C. Denver, Colo.  
 CASE, LUCIUS W. Pomona, Calif.  
 CASILLI, ARTHUR R. Elizabeth, N. J.  
 CASSELMAN, A. J. Camden, N. J.  
 CEFALU, VICTOR. Seattle, Wash.  
 CHAMBERLAIN, ELIZ. M. Bartlesville, Okla.  
 CHERRY, S. L. Clarksburg, W. Va.  
 CLARK, HARRY L. Detroit, Mich.  
 CLARK, J. H. Wyncote, Pa.  
 CLEMMER, J. J. Albany, N. Y.  
 COCHEU, L. F. New York, N. Y.  
 COHEN, FRANK. Quincy, Ill.  
 COHEN, MORTIMER. Pittsburgh, Pa.  
 COLE, R. E. Muncie, Ind.  
 COLLENBERG, H. T. Baltimore, Md.  
 CONNERY, JOS. E. New York, N. Y.  
 COPE, H. E. Detroit, Mich.  
 CORNWALL, L. H. New York, N. Y.  
 CORPER, H. J. Denver, Colo.  
 COSTA-MANDRY, O. G. Porto Rico  
 COVEY, GEO. W. Lincoln, Neb.  
 CRAGG, RICHARD. Rochester, Minn.  
 \*\*CRAIG, CHARLES F. New Orleans, La.  
 †CRAIG, HELEN F. Boise, Idaho  
 CRAWFORD, B. L. Philadelphia, Pa.  
 CRISCITIELLO, M. Pittsfield, Mass.  
 CULBERTSON, CLYDE G. Indianapolis, Ind.  
 \*\*CUMMINGS, HUGH S. Washington, D. C.  
 CUMMINS, W. T. San Francisco, Calif.  
 CURPHEY, THEO. J. Brooklyn, N. Y.  
 CURTIS, STEPHEN HORACE. Troy, N. Y.  
 CUTLER, O. I. Loma Linda, Cal.  
 DALEY, D. F. Kingston, Pa.  
 DALRYMPLE, SID C. Newton, Mass.  
 DARDINSKI, V. Clarendon, Va.  
 DARLINGTON, C. G. New York, N. Y.  
 †D'AUNOY, R. New Orleans, La.  
 DAVIDSOHN, ISRAEL. Chicago, Illinois  
 †DEADMAN, W. J. Hamilton, Ontario, Can.  
 DE COURSEY, ELBERT Washington, D. C.  
 DELANEY, P. ARTHUR. Chicago, Ill.  
 DE LEON, W. Philippine Islands  
 DE WAN, CHARLES H. Sayre, Pa.  
 DICKELMANN, L. E. Oshkosh, Wis.  
 \*DOAN, CHARLES A. Columbus, Ohio  
 DOBOS, EMERIC I. Denver, Colo.  
 \*\*DODDS, E. C. London, W. I.  
 \*DOMANSKI, THADDEUS J. Jersey City, N. J.  
 †DRAKE, C. R. Minneapolis, Minn.  
 \*\*DUKES, CUTHBERT London, E.C. 1, England  
 \*\*DUNGAL, NIELS P. Reykjavik, Iceland  
 DUNLOP, JOSEPHINE N. Pueblo, Colo.  
 \*\*DYKE, S. C. Wolverhampton, Eng.  
 †DYRENFORTH, LUCIEN Y. Jacksonville, Fla.  
 EDGAR, JAMES D. Spokane, Wash.  
 EGGSTON, A. A. New York, N. Y.  
 ELLIOTT, F. P. San Diego, Cal.  
 ELLIS, F. G. Shreveport, La.  
 ELTON, NORMAN W. Buffalo, N. Y.  
 ENZER, NORBERT. Milwaukee, Wis.  
 ERICKSON, MARY J. Thomasville, Ga.  
 ERSKINE, E. B. Jamaica, N. Y.  
 EVANS, NEWTON. Los Angeles, Cal.  
 EXTON, WILLIAM G. New York, N. Y.  
 FALLER, ALBERT. Cincinnati, Ohio  
 FEIN, M. J. Brooklyn, N. Y.  
 FENDRICK, EDWARD. East Orange, N. J.  
 FENNEL, ERIC A. Honolulu, Hawaii  
 FERNAN-NUNEZ, M. Milwaukee, Wis.  
 FETTERMAN, GEO. H. Mayview, Pa.  
 FIDLER, ROSWELL S. Columbus, Ohio  
 FISHER, JESSIE W. Middletown, Conn.  
 †FOORD, ALVIN G. Pasadena, Calif.  
 FORD, JOHN L. Green Bay, Wis.  
 †FOSKETT, H. H. Portland, Oregon  
 FOWLER, KENNETH. Philadelphia, Pa.  
 FOX, HERBERT. Philadelphia, Pa.

- FREEMAN, WM. . . . . Worcester, Mass.  
 FRESHMAN, A. W. . . . . Denver, Colorado  
 FUNK, ERWIN D. . . . . Reading, Pa.  
 GAMBLE, W. G., JR. . . . . Bay City, Mich.  
 GARBER, C. Z. . . . . New York City, N. Y.  
 GARDNER, STELLA M. . . . . Chicago, Ill.  
 GASPAR, I. A. . . . . Rochester, N. Y.  
 GERMAN, WM. M. . . . . Grand Rapids, Mich.  
 \*GETTLER, A. O. . . . . New York, N. Y.  
 GILBERT, RUTH. . . . . Albany, N. Y.  
 GIORDANO, A. S. . . . . South Bend, Ind.  
 GLENN, ROBERT A. . . . . Oakland, Calif.  
 GLIDDEN, H. S. . . . . Tewksbury, Mass.  
 GOEHRING, CARL. . . . . Steubenville, Ohio  
 GOFORTH, JOHN L. . . . . Dallas, Texas  
 GOLDBERG, S. A. . . . . Newark, New Jersey  
 GOLDBLATT, H. . . . . Cleveland, Ohio  
 GOODALE, RAYMOND H. . . . . Worcester, Mass.  
 GORDON, HAROLD. . . . . Louisville, Ky.  
 GOULD, S. E. . . . . Eloise, Mich.  
 †GRAHAM, G. S. . . . . Birmingham, Ala.  
 GRAVES, KENNETH D. . . . . Roanoke, Va.  
 GRAY, JOHN W. . . . . Newark, N. J.  
 GRAY, J. R. T., JR. . . . . Chester, Pa.  
 GRILL, J. C. . . . . Milwaukee, Wis.  
 \*GROAT, WM. A. . . . . Syracuse, N. Y.  
 GRUZHIT, O. M. . . . . Grosse Pointe, Mich.  
 HADEN, RUSSELL L. . . . . Cleveland, Ohio  
 HAGEBUSCH, OMER E. . . . . St. Louis, Mo.  
 HALBACH, R. M. . . . . Toms River, N. J.  
 \*HAMILTON, JOSEPH F. . . . .  
     Memphis, Tenn.  
 HAMMACK, ROY W. . . . . Los Angeles, Calif.  
 HAMMEL, SETH A. . . . . Topeka, Kans.  
 HANAN, E. B. . . . . Bolivar, Mo.  
 HARTMAN, FRANK W. . . . . Detroit, Mich.  
 HARTMAN, GEO. O. . . . . Sharon, Pa.  
 †HASTINGS, LOUIS P. . . . . Hartford, Conn.  
 HATHAWAY, BURR M. . . . . Akron, Ohio.  
 HAUSER, G. H. . . . . New Orleans, La.  
 HEBERT, LOUIS A. . . . . Lake Charles, La.  
 HECK, FRANK J. . . . . Rochester, Minn.  
 HECKER, F. A. . . . . Ottumwa, Iowa  
 HEISE, H. A. . . . . Milwaukee, Wis.  
 \*\*HEKTOEN, L. . . . . Chicago, Illinois  
 HELLWIG, C. A. . . . . Wichita, Kans.  
 HELMBOLD, THEO. R. . . . . Pittsburgh, Pa.  
 HELWIG, F. C. . . . . Kansas City, Mo.  
 HENDERSON, R. C. . . . .  
     Bronx, New York City  
 HENTHORNE, JOHN C. . . . .  
     Rochester, Minn.  
 HERZBERG, M. . . . . Cincinnati, Ohio  
 HILL, LEWIS R. . . . . LaGrange, Illinois  
 HILLKOWITZ, PHILIP. . . . . Denver, Colo.  
 HILLMAN, OLIVER S. . . . . New York, N. Y.  
 HINTON, WM. A. . . . . Boston, Mass.  
 †HIRSCH, EDWIN F. . . . . Chicago, Ill.  
 †HODGES, F. C. . . . . Huntington, W. Va.  
 HOLLINGSWORTH, I. PEMBERTON P. . . . .  
     West Chester, Pa.  
 \*\*HORDER, SIR THOMAS. . . . . London, Eng.  
 HOWARD, LEE. . . . . Savannah, Ga.  
 HOWARD, STACY C. . . . . Ann Arbor, Mich.  
 HOWELL, KATHARINE M. . . . . Chicago, Ill.  
 HUDSON, MARG. G. . . . . Tulsa, Okla.  
 HULL, WAYNE M. . . . .  
     Oklahoma City, Okla.  
 HULSEY, S. H. . . . . Forth Worth, Texas  
 †HUNT, HENRY F. . . . . Danville, Pa.  
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 KAUMP, DONALD H. . . . . Des Moines, Iowa  
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 KELLER, WILBUR F. . . . .  
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     Davenport, Iowa  
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New Orleans, La.

\*SCHERAGO, M.....Lexington, Ky.  
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 STONE, MURRAY C. . . . . Springfield, Mo.  
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 STOUT, SIDNEY E. . . . . Fort Worth, Texas  
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 †THARINGER, E. L. . . . . Milwaukee, Wis.  
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     Fort Dodge, Iowa  
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 THOMPSON, H. A. . . . . San Diego, Calif.  
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 THORNTON, H. C. Indianapolis, Indiana  
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 †TODD, LESTER C. . . . . Charlotte, N. C.  
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 TURNER, GEO. . . . . El Paso, Texas  
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 †VAN ATTA, J. R. Albuquerque, N. M.  
 VAN HECKE, LEANDER J.  
     Wauwatosa, Wis.  
 VAN HORN, H. H. . . . . Harrisburg, Pa.  
 VAUGHAN, S. L. . . . . Buffalo, N. Y.  
 VAUGHAN, WARREN T. . . . . Richmond, Va.  
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 \*VONDERLEHR, R. A.  
     Washington, D. C.  
 \*VON DER LEITH, JOHN F.  
     Jersey City, N. J.  
 VON HAAM, E. . . . . Columbus, Ohio  
 WALKER, THOS. F. . . . . Great Falls, Mont.  
 WALKER, T. T. . . . . Watertown, N. Y.  
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 †WARREN, MORTIMER. . . . . Portland, Me.  
 WARWICK, MARGARET  
     Buffalo, New York  
 \*WATKINS, CHARLES H.  
     Rochester, Minn.  
 WEETER, HARRY M. . . . . Louisville, Ky.  
 WEINGART, JULIUS S.  
     Des Moines, Iowa  
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 WENNER, THOMAS J. Wilkes-Barre, Pa.  
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 WHITE, E. T. . . . . Greenville, Miss.  
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     Beaumont, Texas  
 \*\*WILSON, L. B. Rochester, Minnesota  
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 WURTZ, JOHN G. . . . . Pittsburgh, Pa.  
 \*WYANDT, MISS HELEN. . . . . Omaha, Nebr.  
 \*YAGLE, E. M. . . . . Detroit, Mich.  
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     Pittsburgh, Pa.  
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## NEWS AND NOTICES

Plans for the next convention are being vigorously forwarded. The meeting will be held in San Francisco, California in June with the Tumor Seminar probably on June 9. Definite announcement concerning the exact dates, together with other particulars, will be made in the next issue.

The Local Committee is planning to make the next convention a memorable one.

### MISSISSIPPI VALLEY MEDICAL SOCIETY AWARD

The Mississippi Valley Medical Society offers a cash prize of \$100.00, a gold medal and a certificate of award for the best unpublished essay on a subject of interest and practical value to the general practitioner of medicine. Entrants must be ethical licensed physicians, residents of the United States and graduates of approved medical schools. The winner will be invited to present his contribution before the next annual meeting of the Mississippi Valley Medical Society (September 28, 29, 30, 1938), the Society reserving the exclusive right to first publish the essay in its official publication—the Radiologic Review and Mississippi Valley Medical Journal. All contributions shall not exceed 5000 words, be typewritten in English in manuscript form, submitted in five copies, and must be received not later than May 15, 1938. Further details may be secured from

Harold Swanberg, M.D., Secretary,  
Mississippi Valley Medical Society,  
209-224 W. C. U. Building, Quincy, Ill.

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